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PARASITOLOGY

EDITED BY

GEORGE H. F. NUTTALL, M.D., Ph.D., Sc.D., F.R.S.

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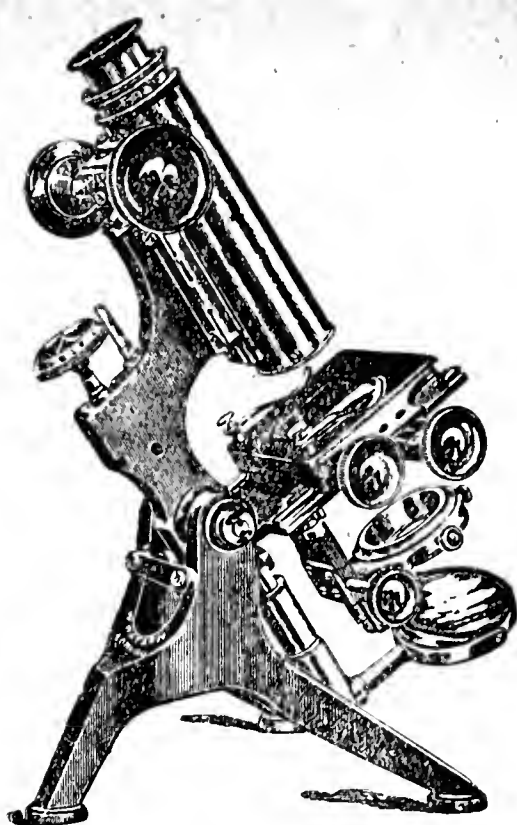
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PARASITOLOGY is published about four times a year. The numbers afterwards are issued in volumes each containing four numbers.

Papers for publication should be sent to Professor GEORGE H. F. NUTTALL, F.R.S., Longfield, Madingley Road, Cambridge. Other communications should be addressed to the University Press, Cambridge.

Papers forwarded to the Editors for publication are understood to be offered to *PARASITOLOGY* alone, unless the contrary is stated.

Contributors receive fifty copies of their papers free. Additional copies, not exceeding one hundred (except in special cases), may be had at cost price: these should be ordered when the final proof is returned.

The subscription price is £2. 5s. per volume (post-free), payable in advance; single numbers 15s. net (double number 30s. net). Subscriptions may be sent to any Bookseller, or to MR C. F. CLAY, MANAGER, Cambridge University Press, Fetter Lane, London, E.C. 4.

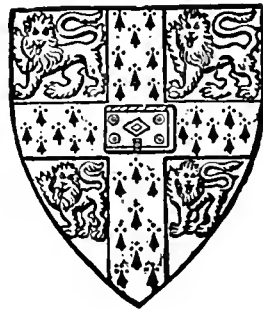
The Cambridge University Press has appointed the University of Chicago Press agents for the sale of *Parasitology* in the United States of America and has authorised them to charge the following prices: Subscription \$9.00 net per volume; single numbers \$3.00 net each, double numbers \$6.00 net each.

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CAMBRIDGE UNIVERSITY PRESS

C. F. CLAY, MANAGER

LONDON: FETTER LANE, E.C. 4



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QUICK PROFESSOR OF BIOLOGY IN THE UNIVERSITY OF CAMBRIDGE

VOLUME XII 1920

CAMBRIDGE

AT THE UNIVERSITY PRESS

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ON COLORATION IN TICKS. II.

BY GEORGE H. F. NUTTALL, F.R.S.

(From the Quick Laboratory, University of Cambridge.)

(With Plates I and II.)

I. COLORATION IN LIVING TICKS.

IN a previous note on coloration in ticks (iv. 1913, *Parasitology*, vi. 49-51, Pl. VII), reference was made to the appearances observed in living and dead specimens, the differences were indicated by coloured figures of *Amblyomma variegatum* (Fabricius) and *A. splendidum* Giebel, and the hope was expressed that future authors would, when practicable, describe the coloration as it is seen by daylight in living specimens.

As a further contribution to the subject, I herewith offer two coloured plates reproducing water colour drawings from living ticks raised by me in Cambridge between 1912 and 1915, the plates having been prepared for incorporation in our *Monograph of the Ixodoidea*. Camera lucida drawings were made by me as the groundwork for these figures all of which were lithographed by the late Mr Edwin Wilson. The latter painted the specimens under my direction, excepting one (Pl. I, fig. 4) which was drawn and coloured by me.

Amblyomma hebraeum Koch. Plate I, figs. 1 and 2 represent living specimens (N. 1732, ♂ and ♀) as they appeared 5. ii. 1914, *i.e.* two months after ecdysis and prior to feeding. The fasting ticks retain this appearance for months. Pl. I, fig. 4 represents the scutum of a ♂ of the same lot after it had remained anchored upon a ram's scrotum for 77 days; it shows that a remarkable change in coloration may occur in ticks of this species that sojourn long upon the host. After 141 days upon the ram the colours in other specimens were found to have become even more intensified, especially the red areas at the sides. I referred but incidentally to this colour change in a previous paper (*Parasitology*, xi. 395, ♂ 5). For an account of the biology of this species, the carrier of Heartwater in Africa, see *Ibid.* vii. 409-419.

Amblyomma gemma Dönitz. Plate I, figs. 5 and 3 represent the scutums of (N. 3016) living unfed ♂ and ♀ specimens as seen on 4. ix. 1914, about two weeks after ecdysis, the specimens having been received from East Africa.

If we compare the scutums of these two species when alive, or when dead for weeks or years and dried or preserved in 70 per cent. alcohol, we may note the following differences (omitting finer details):

Colour changes in the Scutum.

	MALE			FEMALE
	Greater part of pale area	Lateral and marginal pale areas, scapulae	Festoons where pale	Greater part of pale area
In <i>A. hebraeum</i>				
Young, unfed, living	Pale violet	Ochre	Pale yellow	Pale yellow
Ditto, dead, dry	Pale yellow to pale yellowish green	Pale ochre	Pale yellow	Dirty yellow
Old, fed, living	Pale green	Red	Bright yellow	—
Old, fed, dead, dry	Pale green	Red*	Bright yellow	—
Ditto in alcohol (old or young)	Deep violet with green or eoppery sheen at borders	Dark eopper with green sheen	Copper with green sheen	Pink with violet gold and green sheen
In <i>A. gemma</i>				
Young, unfed, living	Salmon, fine bor- der of yellow	Yellow	Pale yellow	Orange, middle violet, posterior margin pale violet
Ditto, dead, dry	Pale orange yellow	Pale orange yellow	Pale yellow	Pale yellow
Ditto in alcohol (old or young)	Copper with blue and green sheen	Copper with blue and green sheen	Pale eopper	Copper with gold and green sheen at borders

* A few specimens had bleached somewhat after 5 years.

The foregoing table serves to illustrate how very differently the ticks' colours appear according to the conditions in which they are examined. In all of the alcoholic specimens the colours are metallic, *i.e.* when viewed by reflected light the coloured parts have the appearance of burnished metal. As stated in my previous paper (*loc. cit.* p. 50), when dried specimens are placed in alcohol the colours become metallic, and vice versa. I do not know of any ticks that show metallic colours when alive.

Dermacentor.

In the species of this genus herein depicted, metallic colours are absent in alcoholic specimens, therefore the colour changes observed in living and variously preserved examples are not so material as in most of the other ornate ticks. In dead specimens, the faint greenish tint seen in the illustration of *D. venustus* may disappear and the darker areas may become blackish in *D. reticulatus niveus*.

Dermacentor reticulatus niveus Neumann. Plate II, figs. 4 and 5 represent (N. 2156) a ♂ and ♀ received in May 1913 from Pina, Spain. I have described the biology of this tick elsewhere, having raised the progeny of the pair here figured (see iii. 1915, *Parasitology*, vii. 421-425).

Dermacentor venustus Banks. Plate II, figs. 1 and 2 represent the ♂ and ♀ of this North American species which is the carrier of Rocky Mountain Fever to man. The specimens figured (N. 1731) were received in June 1912 from Ottawa, Canada. A description of the biology of this species will be found elsewhere (*loc. cit.* pp. 425-430).

Dermacentor variabilis Hunter and Hooker, of which the ♂ is illustrated in Plate II, fig. 3, is likewise an American species. The ornamentation is usually more widely distributed upon the scutum than figured. The illustration is from a solitary adult that I succeeded in raising in Cambridge from (N. 1824) a lot of larvae received in October 1912 from Dr S. Hadwen, the ticks being the offspring of females found 24. vi. 1912 on a horse at Glenora, Manitoba, Canada.

The larvae were placed on a rabbit on 11. x. 1912, in Cambridge. Only 5 gorged larvae were collected, 2 abandoned the host after 4 days, 3 after 5, 7 and 9 days respectively, the rabbit being kept in a room at 15° C. Metamorphosis from larva to nymph took place in 11, 11, 12, 13, 13 days respectively at 21° C. The 5 nymphs were placed upon a rabbit 17–23 days after emergence, the rabbit being kept at 13° C. Only one gorged nymph was recovered after 21 days; it was maintained at 24° C., and after 14 days a ♂ emerged. This ♂ survived unfed for 57 days at 22° C.

A fuller account of the biology of *D. variabilis* has been given by Hadwen (i. 1913, *Parasitology*, v. 234–237).

II. ON THE EFFECT OF TREATMENT WITH CAUSTIC POTASH UPON THE PIGMENTED PARTS OF ORNATE IXODIDAE.

Whilst examining some specimens of *Dermacentor occidentalis* that had been treated with caustic potash and mounted by me in balsam in 1879, I was struck by the circumstance that the creamy white pattern was clearly visible when the transparent specimens were viewed by *reflected* light, whereas specimens of other ornate ticks, similarly prepared, did not show this appearance. After having examined the balsam-mounted material that was available in my cabinet, I selected a series of specimens of ornate ticks from my collection of alcohol preserved material, treated them all alike with caustic potash, and mounted them in balsam.

The ticks of the new series (5. vi. 1915) were placed in 10 per cent. caustic potash solution at ca. 20° C. for 48 hours, then transferred to water for 24 hours, after which they were pricked and pressed between layers of filter paper, cleaned, and placed for 2 days in acidulated (H₂SO₄) water and passed through graded alcohols and clove oil prior to being mounted in balsam.

The examination of the cleared and mounted specimens of the males of different species gave the following results: observations on older material (indicated by o in the list) being included:

Dermacentor	Creamy pattern on scutum, etc. (× = persists, ○ = absent)
<i>albipictus</i> (Packard)	× at scapulae, basis capituli, palps, legs
<i>auratus</i> Supino	× at scapulae, basis capituli, legs
o <i>occidentalis</i> Marx	× × complete
<i>reticulatus</i> (Fabricius)	× × complete in 1 ♂
“ “	× at scapulae, legs, basis capituli, palps in 1 ♂
o <i>venustus</i> Banks	× × complete in 3 ♂♂
<i>rhinocerotis</i> (de Geer)	○

Rhipicephalus

Creamy pattern on scutum, etc. (× = persists, ○ = absent)

<i>dux</i> Dönitz	× traces where ornate
<i>maculatus</i> Neumann	× at posterior spots, slightly at centre
<i>pulchellus</i> (Gerstäcker)	× at scapular and antero-lateral spots

Amblyomma

<i>cajennense</i> Koch	× very slight at light parts
<i>cohaerens</i> Dönitz	○
o <i>cooperi</i> Nuttall and Warburton	○
o <i>decoratum</i> Koch	○
" "	× very slight at scapulae and marginal spots
<i>dissimile</i> Koch	× scapular spots
<i>gemma</i> Dönitz	○
o <i>hebraeum</i> Koch	○
o <i>hirtum</i> Neumann	○
<i>marmoreum</i> Koch	× very slight at scapulae
<i>petersi</i> Karsch	○
<i>pomposum</i> Dönitz	× middle field, antero-lateral fields, trace in first festoons
o <i>splendidum</i> Dönitz	○
<i>testudinarium</i> Koch	× faintly in middle field and basis capituli
o <i>uncatum</i> Nuttall and Warburton	× where spotted posteriorly
o <i>variegatum</i> (Fabricius)	× at emargination in middle field and light scapular spots

Aponomma

<i>decorosum</i> L. Koch	○
<i>exornatum</i> Koch	× at scapulae
o <i>gervaisi</i> (Lucas)	○ or × traces in lateral fields (2 ♂♂)
o <i>gervaisi</i> var. <i>lucasi</i> Warburton	○ in 2 ♂♂

Hyalomma

o <i>monstrosum</i> Nuttall and Warburton	○
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In addition to the foregoing, I have examined the whole of Dr C. W. Stiles' caustic-treated balsam-mounted *Dermacentor* material, comprising 58 adults, all of which shows the creamy white coloration previously referred to when viewed by reflected light. Stiles' material comprises specimens labelled *Dermacentor electus* Koch (2 ♂ 1 ♀), *D. occidentalis* Marx (1 ♂ 3 ♀), *D. andersoni* Stiles (9 ♂ 39 ♀) and *Dermacentor* sp. (2 ♂ 1 ♀), all of which are North American species.

The foregoing records show that after caustic potash treatment the creamy markings only appear to persist clearly and completely in certain members of the genera *Dermacentor* and *Rhipicephalus*. Under a high magnification the chitin in the region of the creamy markings appears finely granular as if it included particulate matter. *Dermacentor rhinocerotis*, which yields different results to the other members of its genus that have been examined, bears dull yellowish spots on its scutum in dried specimens, whereas in well-preserved alcoholic specimens the spots have a yellow or pale pink metallic lustre; neither before or after caustic treatment does the species show a creamy ornamentation. The three species of *Rhipicephalus* examined are the only ones that are at present known to be ornate.

In the remaining genera, when specimens are treated with caustic, all

remains of pattern either disappear or but traces remain in certain regions perhaps partly because these are less accessible to contact with the caustic.

On the other hand, where the scutum is dark brown or black, as in certain thicker and well-defined portions where "dark markings" are referred to in descriptions, these markings persist after caustic treatment. It is interesting to note that the bright orange spot occupying the centre of the scutum in *Amblyomma splendidum* ♂ (illustrated by me in *Parasitology*, vi. Pl. VII, figs. 5 and 6) appears to offer an exception in that it corresponds to a dark spot in caustic-treated specimens, whilst no similarly situated dark spot is observable in the scutums of the closely allied species *A. hebraeum*, *A. cohaerens*, etc.

SUMMARY AND CONCLUSIONS.

The coloration of *Amblyomma hebraeum*, *A. gemma*, *Dermacentor venustus*, *D. variabilis* and *D. reticulatus niveus*, as seen in living examples, is depicted for the first time, and the desirability of recording the colours of ornate ticks when alive is indicated.

A remarkable change in colour in living specimens of *A. hebraeum* is described and figured, this change taking place after a prolonged sojourn (74–141 days) upon the host. Such a colour change has not hitherto been observed in ticks. The difference in colour is seen in dead dried specimens but is not appreciable in those preserved in alcohol.

Since the immature stages of ornate ticks are inornate, and the colours change in adults of some species during prolonged periods of parasitism, it is evident that the coloration in adults must depend upon the accumulated products of metabolism beneath the chitinous exoskeleton, the regional distribution of colour depending upon special metabolic functions taking place in corresponding parts of the tick. The ornamental colour-producing layer can be scraped away from the underside of the scutum in most ornate ticks and it is removable from such ticks by the use of caustic potash.

The whitish or creamy coloration that is so characteristic of most species of *Dermacentor* and the three ornate species of *Rhipicephalus* that are known to science, appears on the other hand to depend largely upon inclusions or structural changes within the chitin itself, whence the persistence of the creaminess seen by reflected light in the caustic-treated ticks. Similar, but less distinct, appearances may be seen in ticks belonging to other genera. *Dermacentor rhinocerotis*, which does not exhibit creamy coloration but only dull yellowish spots when dry, is totally decolorized by caustic potash, whilst contrary to most species of its genus it shows metallic coloration in well-preserved specimens in alcohol.

The examination of caustic-treated specimens was carried out upon 31 species of ornate ticks, *i.e.* *Dermacentor* (8), *Rhipicephalus* (3), *Amblyomma* (15), *Aponomma* (4) and *Hyalomma* (1 species).

The coloration and creamy ornamentation, herein distinguished, appear

to be confined to the thinner portions of the exoskeleton. That the characteristic dark markings correspond to thicker and more darkly chitinated portions of the scutum is demonstrable by dissections, caustic-treated, or sectioned specimens; this being especially evident for instance in *Amblyomma*. The orange spot on the scutum of *A. splendidum* offers an exception.

The optical and chemical study of coloration in ticks deserves further investigation.

DESCRIPTION OF PLATES I AND II.

All of the specimens were viewed by daylight and magnified about $\times 12$ under a Zeiss binocular Dissecting Microscope.

PLATE I.

- Fig. 1. *Amblyomma hebraeum* Koch. Living unfed ♂, 2 months old (reckoned from last ecdysis).
- Fig. 2. *A. hebraeum* Koch. Living unfed ♀, 2 months old.
- Fig. 3. *A. gemma* Dönitz, scutum of living unfed ♀, about 2 weeks old.
- Fig. 4. *A. hebraeum* Koch, scutum of living ♂ after sojourn of 77 days on the host.
- Fig. 5. *A. gemma* Dönitz, scutum of living unfed ♂, about 2 weeks old.

PLATE II.

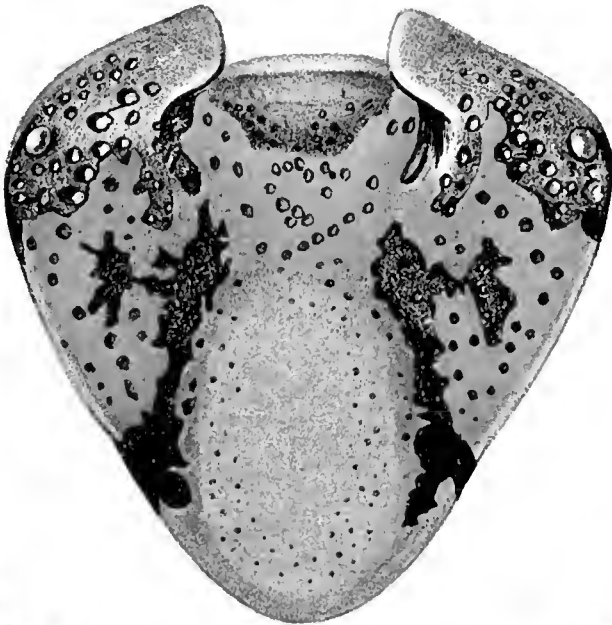
- Fig. 1. *Dermacentor venustus* Banks, living ♂.
- Fig. 2. *D. venustus* Banks, living ♀.
- Fig. 3. *D. variabilis* (Say), living ♂.
- Fig. 4. *D. reticulatus niveus* Neumann, living ♂.
- Fig. 5. *D. reticulatus niveus* Neumann, living ♀.



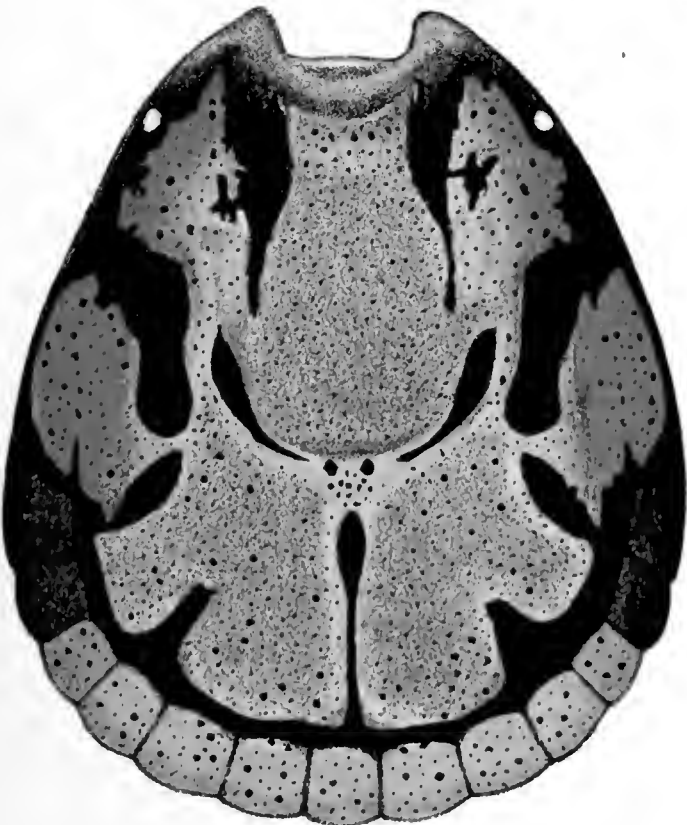
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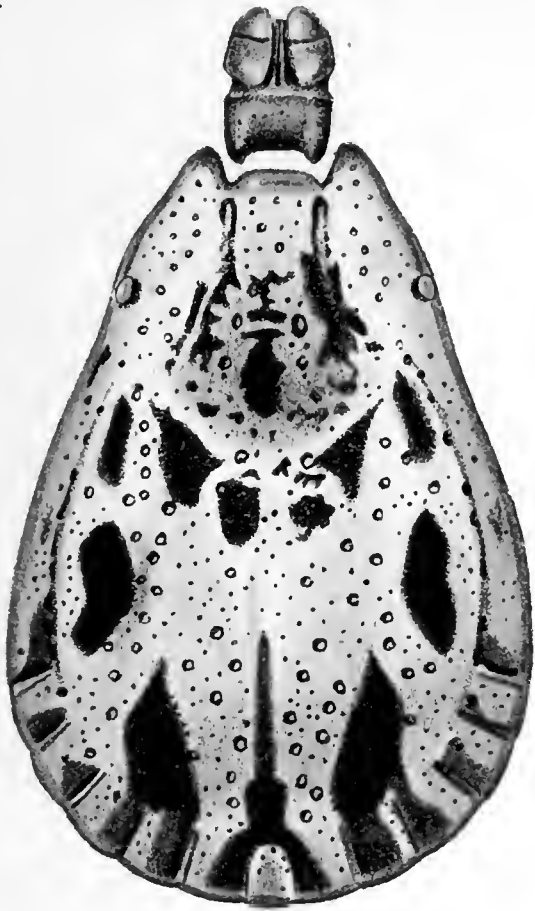


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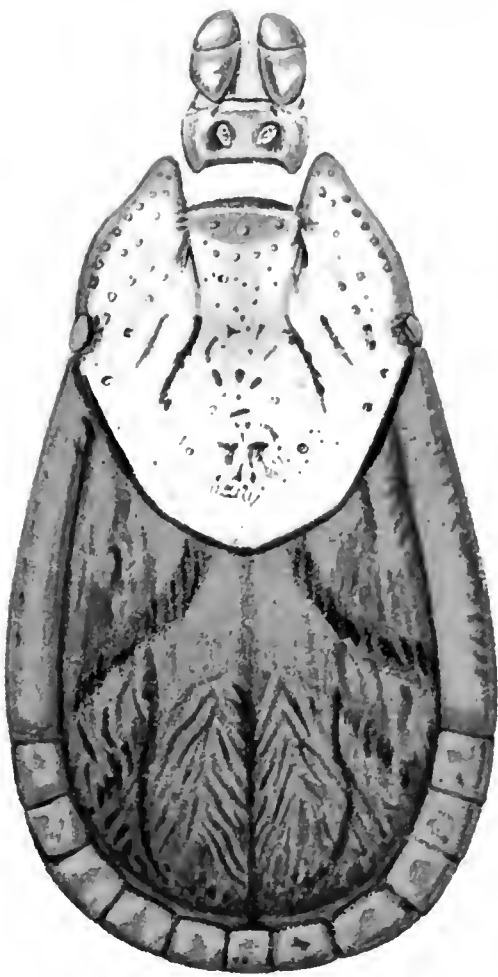


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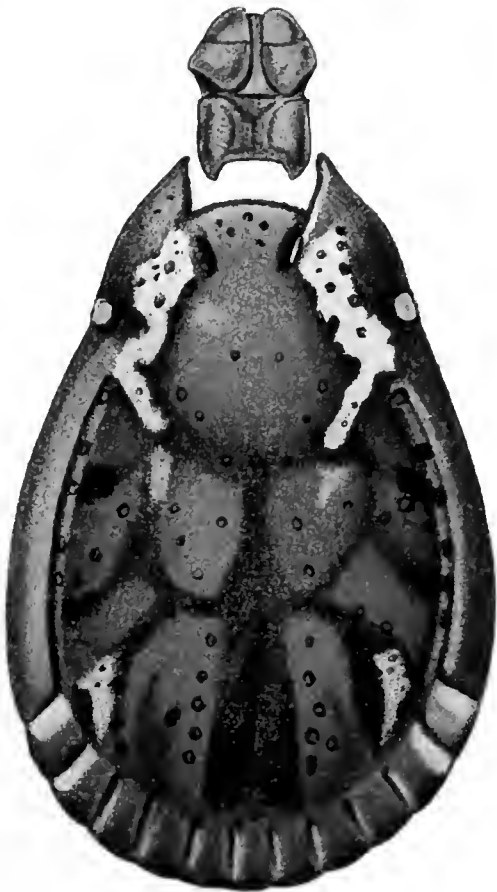
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REGENERATION OF THE MOUTHPARTS AND LEGS IN TICKS.

ARGAS PERSICUS, *AMBLYOMMA HEBRAEUM*
AND *HYALOMMA AEGYPTIUM*.

BY GEORGE H. F. NUTTALL, F.R.S.

(From the Quick Laboratory, University of Cambridge.)

(With 6 Text-figures.)

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INTRODUCTION.

THE only authors who have concerned themselves with regeneration in Ixodoidea are Hindle and Cunliffe¹, who, at my suggestion, carried out experiments wherein they mutilated the legs of immature *Argas persicus* and observed the effects of various operations upon the development of the limbs in succeeding stages. I had laid it down as a part of the programme that these authors should also determine if the mouthparts of immature ticks are capable of being regenerated, but they confined their attention to the regeneration of the legs, it being more difficult to deal with the mouthparts especially in small larvae. Ticks are liable to be mutilated in nature by being forcibly removed from the host, whereby their mouthparts are subjected at times to

¹ Hindle, E. and Cunliffe, N. (i. 1914). Regeneration in *Argas persicus*. *Parasitology*, vi. 353-371, 4 text-figures.

considerable injury. These may remain anchored in the host's skin, when the tick's body is torn away, and every grade of injury to the mouthparts is observable in ticks that are collected in the field.

METHODS.

In all cases the ticks under experiment were operated upon by me as soon as practicable, *i.e.* an hour or two, after they had fed to repletion and had dropped off the host. A requisite number having been selected for operation, individual ticks were transferred successively to a holder that kept them in a convenient position during the brief operation which took place upon the stage of a Zeiss binocular microscope.

The holder consisted of a glass photographic plate of small size upon which a band of plasticine adhered. The tick was placed on the plasticine with its mouthparts or legs that were to be amputated projecting out over the edge of the plasticine, and a second band of plasticine, covered with a piece of fine linen, was laid upon the first and pressed down sufficiently to fix the tick in position. The tick was therefore held as it were in a vice, a soft one, the gentle grip of which could be regulated during the operation and easily readjusted if the tick shifted. The piece of linen laid on the plasticine prevented its sticking to the operator's fingers during the manipulations.

A polished scalpel was held in the left hand with the blade beneath the tick's capitulum (or legs) so that the steel could be cut down upon as on a plate by means of a fine needle held in the right hand and ground to an oblique chisel edge. Great care was taken to avoid bruising the parts or pulling them about and to make a clean cut expeditiously.

The amputated parts adhered as a rule to the scalpel through the little coelomic fluid that escaped from the tick's wound when the parts were cut, rarely did the amputated parts spring away and get lost. They were collected after each operation and mounted in balsam as follows:

A number of slides were prepared by scratching two rows of small circles (ca. 6 mm.) upon them with a writing diamond, a circle punched in a piece of card and held firmly against the slide serving as a guide to the diamond point. The slide was now reversed so that the diamond circles were on the under surface and afterwards clearly visible through the balsam mount on the upper surface, on which, at the margin of the slide and close to each circle, a number was scratched, the number corresponding to that of the tick that was to be operated upon. The slide was cleaned, and a minute drop of water was placed over the centre of a diamond circle and brought into focus upon the stage of a second dissecting microscope. The amputated parts of the tick were transferred on the point of the operating needle to the drop of water and this was allowed to dry. When the series of circles was occupied by amputated parts and these had dried, a small drop of xylol was placed upon them and this was followed by a drop of balsam. Ordinary cover-glasses cut in four yielded squares of a size that sufficed to cover the circles.

These permanent preparations, ten or more per slide, served as accurate checks to graphic notes that were made of each operation. The skins of the ticks as they moulted after operation, or the ticks themselves, were mounted serially in a similar manner, air being excluded from the mounts by taking the usual precautions. The diamond circles greatly facilitate the finding of minute objects mounted within their contours¹.

The ticks, after operation, were confined separately in numbered tubes standing in racks within a thermostat at 30° C., whence they were taken daily for inspection; the cast skins were periodically removed and mounted in the manner previously described.

The *abbreviations used in the tables* that follow are

R and L denoting Right and Left in respect to the parts that were amputated or left untouched.

— (a dash) denotes that no operation took place on the parts indicated.

The degree of amputation undergone by the several parts is denoted in different ways:

(a) by *fractions* of the length of the part (hypostome, palps) from apex to base, *i.e.* 1/2, 1/3, etc.

(b) by stating the *number of articles* that were removed wholly or partly, reckoning the basal article as 1 (palps have 4 articles 1-4) or by recording the number of distal articles removed (legs have 6 articles including the coxa).

(c) Operations where digits were removed without appreciable injury to the *shaft* of the chelicerae, are indicated by *cut d*; where the shaft was cut across, the amount thereof removed is given in lengths of digit taken as a measure, thus *cut 4 l* denotes that the portion of shaft removed with the digit was four times the length of the digit.

¹ I have devised another simple method of mounting large numbers of small objects in series. The method consists in mounting each small object in a numbered cell in balsam. To do this, cardboard of suitable thickness is cut in oblongs that are shorter (to leave room for a label) and nearly as wide as a slide. Two rows of circular holes (ca. 6 mm.) totalling 10-12 in number, are neatly cut in the card with a sharp hand-punch and serial numbers are written in Indian ink on the card beside the holes. Before use, the holed cards are immersed in xylol for 24 hours and afterwards in balsam for 24 hours; they are then transferred to a slide, the objects are placed in the holes and these are filled with balsam. A coverglass, of a size to match the card, having been dipped in xylol, is at once placed in position, the use of xylol helping to exclude air bubbles. The method is very convenient, economical, and readily lends itself to modifications.

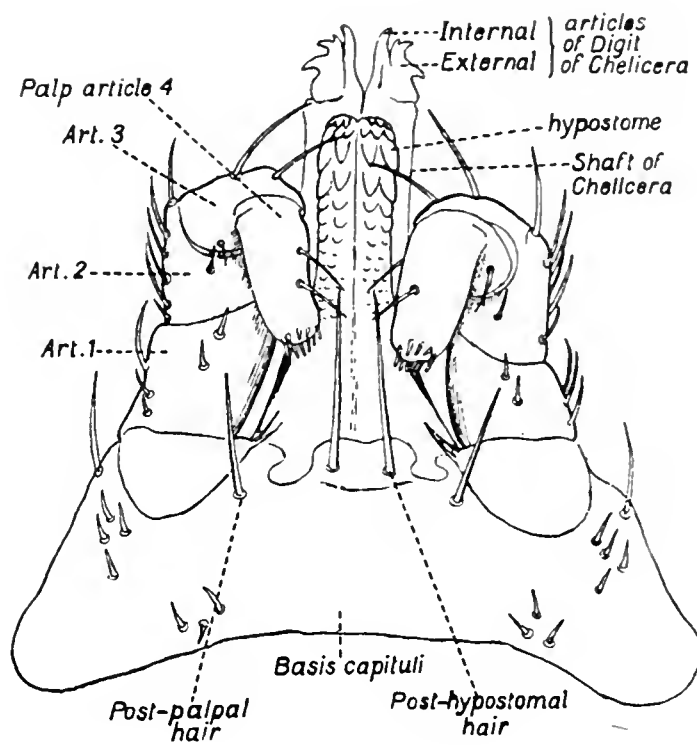


Fig. 1. *Argas persicus* ♂. Capitulum in ventral aspect, giving nomenclature of parts enumerated in the text (Nuttall, 1908).

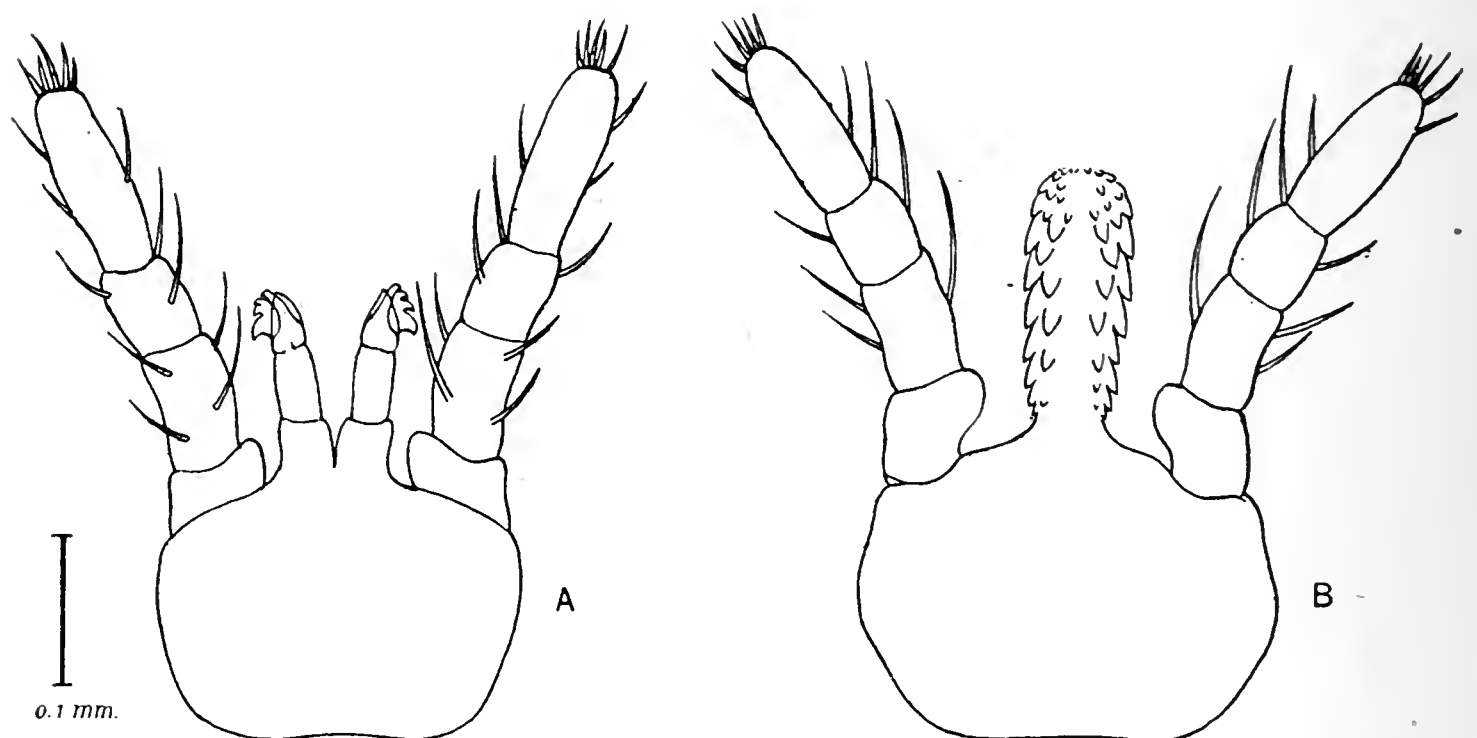


Fig. 2. *Argas persicus* larva. (A) Capitulum in dorsal and (B) in ventral aspect (Orig.).

I. REGENERATION AFTER AMPUTATION OF THE MOUTHPARTS.

(Experimental Records.)

1. *Argas persicus*.(a) *Operations on the larva and their effect on the first-stage nymph.*

Twenty-two larvae were operated upon after feeding on a fowl. Six (Ticks 3, 6, 13, 15, 17, 19) failed to undergo metamorphosis or died soon after operation. Twelve larvae were operated upon on 22. ii. 1915 and ten on the next day. The following record relates to the sixteen specimens that survived and moulted to first-stage nymphs.

Argas No.	Nature of operation on larva			Resultant effect on first-stage nymph		
	Palp articles removed	Hypostome length removed	Chelicerae	Palps showed stumps at article	Hypostome	Chelicerae d = digit
1	R 1-4 L 4	1/3	R — L cut*	R base L 1	perfect	L d perfect
2	R — L 2-4	1/4	R cut L cut	R 2† L 1	perfect	R and L d deformed
4	R 2-4 L 1-4	at base	R — L cut 1 l	R 3 L 3	regen. but deep emarg.	L d deformed
5	R 2-4 L 2-4	2/3	R — L —	R 1 (fig. 4 B) L 1	perfect	—
7	R — L 2-4	at base	R — L —	R deformed† L 1	deformed (see fig. 4 G)	—
8	R 2-4 L —	3/5	R — L —	R 1 L 1	perfect	—
9	R 2-4 L 2-4	1/4	R cut L cut	R and L 1 (fig. 4 D)	perfect	R d deformed (fig. 4 K) L d ?
10	R 2-4 L 2-4	at base	R cut 1/2 l L cut 1 l	R and L 2 (fig. 4 C)	regen. but some asymmetry	R d distinctly, and L d slightly deformed (fig. 4 J)
11	R 3-4 L 3-4	2/5	R — L —	R 2 L 2	perfect	—
12	R 3-4 L 3-4	1/4	R cut L cut	R and L 3 (fig. 4 E)	perfect	—
14	R — L —	1/4	R cut 4 l L —	—	regen. slight asymmetry	R d not regen. (see fig. 4 M)
16	R — L —	2/5	R cut 1 l L cut 1 l	R deformed but of normal length†	regen. slight emarg.	R d deformed L d deformed
18	R 2-4 L 2-4	at base	R cut 1 l L cut 1 l	R 1 L 1	perfect	R d deformed R d distinctly deformed
20	R — L —	near base	R cut d L cut 1 l	—	perfect	R d deformed L d deformed, useless
21	R — L —	2/5	R cut 1 l L cut 1 l	—	regen. slight asymmetry (fig. 3 A)	R d slightly deformed L d „ „
22	R 2-4 L crushed?	2/5	R cut L cut	R 1 L 2†	do.	R d useless (fig. 4 L) L d slightly deformed

* Owing to loss of some amputated parts of chelicerae it is impossible in all cases to specify how much was removed.

† The tick must have been unwittingly injured at operation.

(b) Operations on first-stage nymphs and their after effects.

Twenty-one first-stage nymphs were operated upon on 15-16. iii. 1915, after feeding on a fowl.

Argas No.	Nature of operation on first-stage nymph			Resultant effect on after stages				
	Palp articles removed	Hypostome cut at	Chelicerae digits	Moulted on day and month (1915)	Palps showed stumps at article	Hypostome	Chelicerae digits	Subsequently fed, and developed perfectly, moulted on
28	R — L 2-4	base	R — L —	4. iv.	R L 4	perfect	—	7. v. ♂
29	R 2-4 L —	base	R hurt? L —	29. iii.	R stumpy	perfect	perfect	28. v. ♀
30	R hurt? L —	base	R cut d L —	4. iv.	R 2	regen., emarg.	R perfect	1. vi. ♀
31	R 3-4 L —	base	R — L —	1. iv.	R stumpy	perfect	—	10. v. ♂
32	R — L 2-4	base	R — L —	29. iii.	L short	perfect	—	8. v. 3rd-st. nymph
33	R 3-4 L —	base	R hurt? L —	26. iii.	R 3	perfect	—	8. v. ♀
34	R — L —	base	R cut 1 l L cut	30. iii.	—	perfect	R slightly def. L perfect?	8. v. ♀
35	R — L —	base	R cut d L —	29. iii.	—	regen., emarg.	R. v. slightly deformed	26. v. 3rd-s nymph, refused to feed
36	R — L —	base	R cut 1 l L cut d	1. iv.	—	perfect	R slightly def. L deformed, small	11. v. ♀
37	R — L —	base	R cut d L —	30. iii.	—	normal?	R (lost)	4. vi. ♀
38	R — L —	base	R cut 1 l L cut 1 l	30. iii.	—	slightly emarg.	R and L (lost)	28. v. ♂
39	R — L —	base	R — L —	30. iii.	—	do.		28. v. ♂
40	R — L —	base	R cut 2 l L cut 1 l	2. iv.	—	perfect	R d deformed L d normal	2. vi. ♂
41	R — L —	base	R cut d L —	30. iii.	—	perfect	R d deformed	10. v. ♀
42	R — L —	base	R cut 1 l L cut d	2. iv.	—	perfect	R and L slightly deformed	11. v. ♀
43	R — L 2-4	base	R cut d L —	29. iii.	L regen. but short	perfect	R d perfect	20. vi. ♂
44	R — L 4	base	R — L cut d	30. iii.	do.	perfect	L d deformed	10. v. ♂
53	R 2-4 L —	base	R cut d L —	3. iv.	R few sensory hairs at tip	perfect	R d deformed	22. vi. ♀
55	R — L 2-4	base	R cut 1 l L cut 1 l	1. iv.	L imperfect	perfect	R and L d slightly deformed	1. vi. ♂
59	R 3-4 L 1-4	—	R cut d L —	1. iv.	R 4 L 2	regen., emarg.	R and L slightly small	31. v. ♂
62	R 2-4 L 2-4	base	R cut d L cut d	2. iv.	R and L small?	perfect	R and L deformed	12. v. ♀

Amputations through the basis capituli.

Ten first-stage nymphs had the basis capituli cut through transversely on 15. iii. 1915. Only four survived this severe operation which was accompanied by a considerable loss of coelomic fluid.

<i>Argas</i> No.	Cut across basis capituli	After effects on 2nd-stage nymph
50	Cut diagonally near base of palps, including a long piece of shaft and digits of chelicerae.	19. iv. 15. Found partly moulted, feeble. Exuviae were carefully removed and found palps absent; hypostome represented by a smooth stump; chelicerae digitless, the tip of one sheath toothed, the other rounded.
51	Cut similarly to No. 50 but more basally.	19. iv. Found partly moulted, feeble, without capitulum, hypostome or palps, whilst two flattened digitless cheliceral sheaths protruded like fingers from a glove.
54	Cut similarly to the last.	5. iv. Moulded and died on 30. iv. Palp absent on the right side and reduced to a thin finger-like process on the left side; hypostome absent; chelicera of left side with its digit very small, the digits on both sides deformed (see Fig. 3 B and B').
60	Cut across anteriorly, a good portion of cheliceral shaft being included.	1. iv. Found moulting, thin and weak; it was helped to free itself. The tick died on 28. iv. Whilst it possessed neither hypostome nor palps, the chelicerae appeared normal.

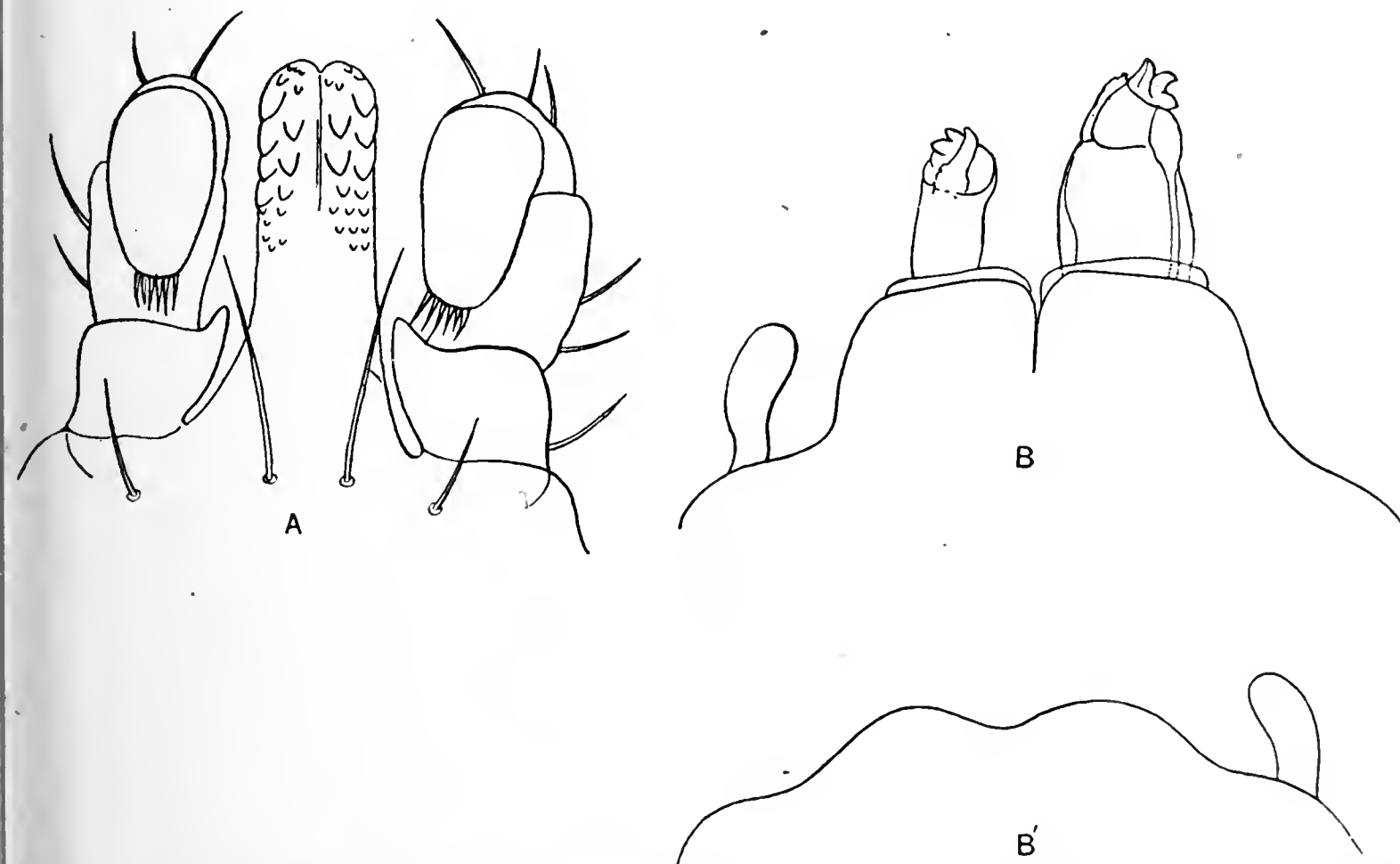


Fig. 3. *Argas persicus* nymphs. (A) Capitulum of 1st-stage nymph which had its hypostome and digits removed when in the larval stage (*Argas* 21); ventral aspect. (B) Capitulum of a 2nd-stage nymph which had its basis capituli cut across when in the first nymphal stage (*Argas* 54); dorsal aspect. (B') Ventral aspect of the same, showing absence of hypostome, one palp reduced to a finger-like process and the other absent, digits deformed.

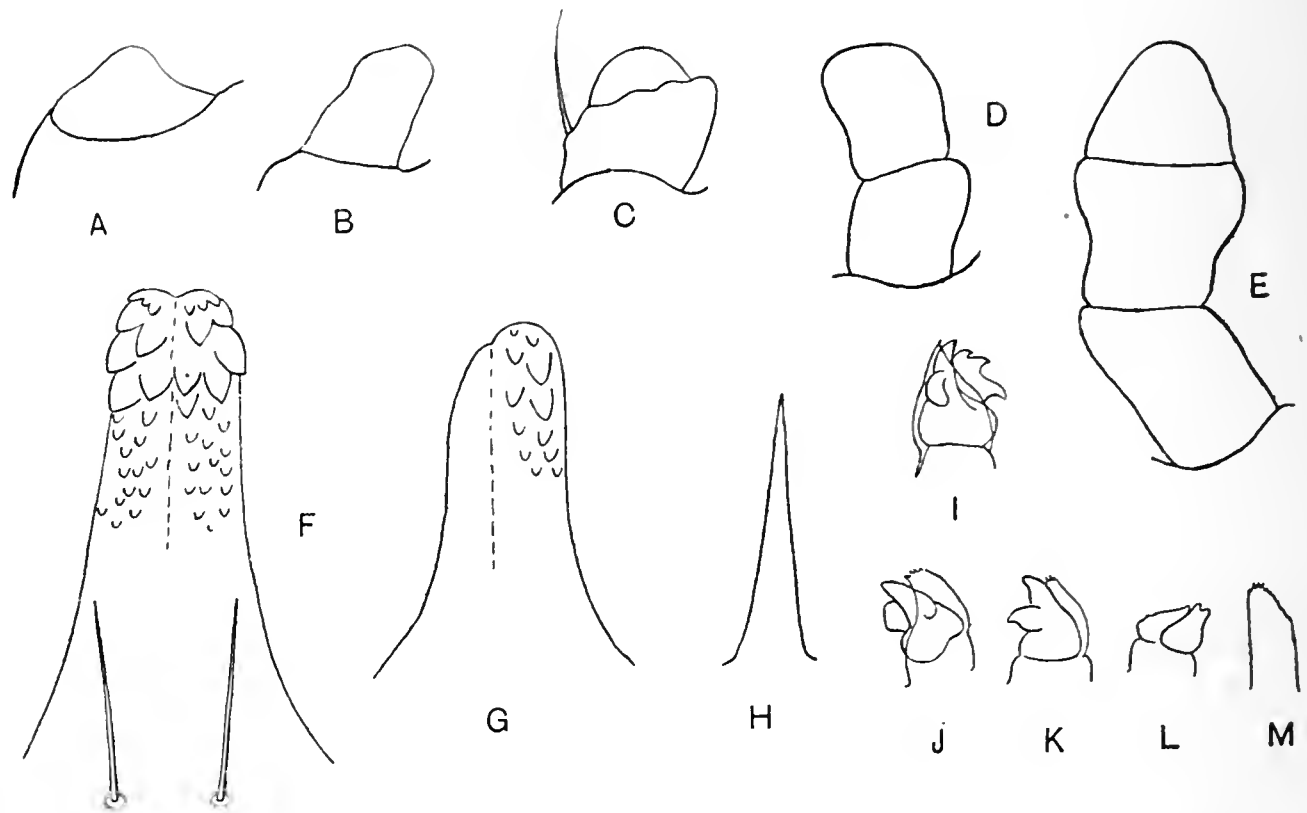


Fig. 4. *Argas persicus* nymphs. Illustrating effects of mutilations inflicted in preceding stages:

- (A) *Argas* 22, right palp in ventral aspect. First-stage nymph operated upon as a larva. Article 1 partly regenerated.
- (B) *Argas* 5, ditto. The left palp closely resembled the one figured at (A). Partial regeneration of article 1.
- (C) *Argas* 10, ditto. The left palp presented a similar appearance in this specimen. Regeneration of article 1 and a stump of article 2.
- (D) *Argas* 9, ditto. The left palp was similarly formed. Articles 1 and 2 regenerated.
- (E) *Argas* 12. Left palp in ventral aspect. First-stage nymph that had been operated upon as a larva. Articles 1 and 2 regenerated but malformed; article 3, partly regenerated, forms the stump.
- (F) Hypostome of *normal* 1st-stage nymph.
- (G) Deformed hypostome of *Argas* 7, a 1st-stage nymph that had been operated upon as a larva.
- (H) Much deformed hypostome of *Argas* 67, a 3rd-stage nymph that had been operated upon as a 2nd-stage nymph. The structure forms a toothless spine.
- (I) Digit of *normal* 1st-stage nymph in dorsal aspect.
- (J-K-L) Deformed digits of 1st-stage nymphs, in dorsal aspect, as they appeared in *Argas* 10, 9, 22 that had been operated upon as larvae.
- (M) Digitless end of right chelicera in *Argas* 14, a 1st-stage nymph that had been operated upon as a larva.

(c) *Operations on second-stage nymphs and their after effects.*

Fifteen second-stage nymphs were operated upon on 29. iv. 1915 after feeding on a fowl. The following record relates to ten of these that survived and moulted to adults or third-stage nymphs which were subsequently raised to adults.

Argas No.	Nature of operation on second-stage nymph			Moulted on	Resultant effect on after stages
	Palp articles removed	Hypostome cut off at	Chelicerae		
63	R 2-4 L —	base	R and L cut d	18. v. 15	perfect ♂.
65	R and L 2-4	base	R cut d L —	18. v. 15	perfect ♂.
67	R at base L at base	base	R cut d L cut	25. v. 15	A 3rd-stage nymph with palps regenerated, hypostome a toothless spine (fig. 4 H), digits normal. Refused to feed and finally died.
68	R 2-4 L 1-4	base	R and L —	15. v. 15	♀, normal but for asymmetric emarginate hypostome. Fed 28. v.
70	R 2-4 L 1-4	base	R and L cut	17. v. 15	3rd-stage nymph normal but for somewhat asymmetric emarginate hypostome and rather small teeth on the external article of digits. Fed on 28. v.
74	R 2-4 L 2-4	base	R and L cut d	14. v. 15	3rd-stage nymph, perfect; fed 3. vi. and moulted 17. vi. as a normal ♀.
75	R 2-4 L 3-4	base	R cut 1 l L —	22. v. 15	3rd-stage nymph, perfect.
76	R 2-4 L 2-4	1/2	R cut L —	18. v. 15	3rd-stage nymph, perfect.

Amputations through the basis capituli.

Three second-stage nymphs had the basis capituli cut across on 29. iv. 15, and two survived the operation:

Argas No.	Cut across basis capituli		After effects on third-stage nymph
72	Cut diagonally from behind one palp to base of other and through digits and pieces of cheliceral shafts.	Tick moulted on 19. v.	Fed on 28. v. The tick was perfectly formed, the external article of the digits was somewhat small.
73	Cut midway across its length and through digits and cheliceral shafts to thrice the length of digit.	Tick moulted on 25. v.	Refused to feed 28. v. Its digits were deformed, the hypostome was absent, the palps merely represented by small protrusions. It survived in this condition until 13. v. 1919 (<i>i.e.</i> four years unfed) when it was killed and preserved.

2. *Amblyomma hebraeum*.(a) *Operations on the larva and their effects on the nymph.*

Thirteen larvae were operated upon on 27. vii. 1915, after they had fed to repletion upon a hedgehog and dropped off the host.

<i>Amblyomma</i> larva No.	Nature of operation on larva			Regeneration observed in nymph
	Palps	Hypostome cut away	Chelicerae digits	
1	R and L —	at base	L cut 4 l	perfect
2	R and L —	at base	R and L cut 3 l	perfect
3	R and L —	at base	R and L cut 3 l	perfect
4	R and L —	1/2	R cut 2 l L cut 1/2 l	perfect
5	R and L —	at base	R cut 3 l	R chelicera shaft 1/4 shorter than normal, otherwise perfect
6	R cut at base L cut near base	1/2	R cut 1/2 l L cut 2 l	perfect
7	R cut at base L —	3/4	R and L cut 2 l	perfect
8	R and L —	3/4	R cut 1 l L cut 2 l	perfect
9	R — L cut 1/2	at base	R cut 2 l L cut 2 l	perfect
10	R — L at base	at base	R cut 2 l L —	perfect

(b) *Operations on the nymph and their effects on the adult.*

Ten nymphs were operated upon on 3. viii. 1915, after feeding to repletion on a hedgehog.

<i>Amblyomma</i> nymph No.	Nature of operation on nymph			Regeneration observed in adult ♂ or ♀
	Palp articles cut away	Hypostome cut across	Chelicerae length of shaft cut in terms of digit length	
1	R and L —	at base	R and L cut 4 l	♂ perfect
2	R and L —	at base	R cut 2 l L cut 3 l	♂ perfect
3	R and L cut 2-4	under base	R and L cut 2 l	♀ perfect
4	R — L cut 2-4	at base	R and L cut 1 l	♀ perfect
5	R and L 2-4	at base	R cut 1 l L cut 3 l	♀ perfect
6	R cut 3-4 L cut 2-4	at base	R — L cut 2 l	♀ perfect
7	R cut 2-4 L cut 1-4	at base	R and L cut 2 l	♂ perfect

3. *Hyalomma aegyptium*.

Operations on the nymph and their effects on the adult.

Seventeen nymphs were operated upon on 1. vi. 1915, after feeding and dropping from a hedgehog. They all moulted between 17 and 22. vi., except No. 17 which moulted on 24. vi. 15.

<i>Hyalomma</i> No.	Nature of operation on nymph			Regeneration observed in adult ♂ or ♀
	Palp articles removed	Hypostome cut	Chelicerae	
1	R and L —	nr base	R and L cut 2 l	♀ right internal article of one digit slightly deformed, otherwise perfect
2	R and L —	nr base	R cut 1 l	♀ perfect
3	R and L —	nr base	R cut 2 l L cut 1 l	♀ perfect but for deformity of external article of right digit
4	R and L —	nr base	R and L cut 2 l	♀ perfect
5	R and L —	at base	R cut 3 l	♀ perfect
6	R cut 2-4 L —	nr base	R and L cut 2 l	♂ perfect
7	R cut 1-4 L —	nr base	R cut	♀ perfect but for deformed R internal article of digit
8	R and L cut 2-4	at base	R and L cut 4 l	♀ R and L digits badly deformed, rest normal
9	R and L —	nr base	R cut	♀ perfect
10	R and L —	nr base	R and L cut 1 l	♀ perfect

II. REGENERATION AFTER AMPUTATION OF THE LEGS.

(Experimental Records.)

The experiments of Hindle and Cunliffe (cited on p. 7) on the mutilation of the legs in *Argas persicus* may be summarized as follows:

When the legs of a freshly gorged larva are amputated, they are not regenerated or are imperfect in the first-stage nymph. If the larva is operated upon whilst on the host, that is 2-3 days *before* it would drop off gorged if left unmolested, the legs may at times be regenerated after the tick abandons the host. This difference in the behaviour of the larva under the two conditions specified is attributed to nymphal development proceeding within the larva whilst it is upon the host and developing nymphal tissues being injured when gorged larvae are operated upon. First-stage nymphs that were mutilated in the larval stage, when raised, without further operative interference to second-stage nymphs, were found to have regenerated the amputated limbs although these were usually of subnormal size whilst perfectly formed; after a further moult, the ticks became normal. Experiments were also made with nymphs. It was found that leg regeneration occurred in all immature stages where amputation took place sufficiently long before moulting, but the legs were usually of subnormal size.

In my experiments the legs were amputated in immature stages of *Amblyomma hebraeum*, *Hyalomma aegyptium*, and in a few *Argas persicus* for purposes of comparison:

1. *Argas persicus*.

(a) *Operations on the larva and their effect on the first-stage nymph.*

Legs were amputated in three larvae (Nos. 23, 24, 26) on 23. ii. 15. In No. 23 legs I–III on the right side had 4, $3\frac{1}{2}$, and 4 distal articles cut off respectively; in No. 24 leg III had $2\frac{1}{2}$ articles removed; in No. 26 leg I had 4 articles removed.

In none of these larvae were the limbs regenerated in the first-stage nymph, all of the limbs ended in stumps corresponding to the seat of amputation in the larva. No. 26 moulted as a first-stage nymph on 17. iii., it fed on 25. iii., and on 19. iv. it was found to have moulted with all its legs normally formed.

(b) *Operations on the first-stage nymph and their effect on the second-stage nymph.*

The legs of the right side were amputated in three first-stage nymphs (Nos. 45–47) on 25. iii. 15. In No. 45 legs I and IV had 5 articles removed; in No. 46 legs I and II had 5 and 3 articles removed respectively; in No. 47 legs I and III had 4 articles removed. The three ticks moulted to second-stage nymphs on 30. iii.–2. iv. and fed normally on 28–30. iv.

In the second nymphal stage, No. 45 had legs I and IV of subnormal size, and No. 46 had legs I and II smaller than the corresponding legs on the side that had not been mutilated. Through an oversight No. 47 was allowed to moult and the moulted legs were lost so that its condition escaped observation.

After further feeding, Nos. 45 and 47 moulted to third-stage nymphs whilst No. 46 emerged as a normal ♂, skipping the third nymphal stage which is more commonly omitted in normal ticks of this species. No. 47 gave rise to a ♀. In all of these stages the ticks appeared perfectly formed, no difference in size between corresponding legs being observable.

2. *Amblyomma hebraeum*.

(a) *Operations on the larva and their effect on the nymph.*

Legs were amputated in three larvae (Nos. 11–13) on 27. vii. 15 as follows: In No. 11 the greater part of legs II and III was removed; in No. 12 leg I had 5 articles removed; in No. 13 leg III had 5 articles removed.

Examined after they had moulted, the nymphs were found to have regenerated their legs perfectly, the previously mutilated limbs not being smaller than normal.

(b) Operations on the nymph and their effect on the adult.

Legs were amputated in three nymphs (Nos. 8-10) on 3. viii. 15 as follows: In No. 8 legs III and IV had 3 and 4 articles removed respectively; in No. 9 legs II and III lost 3 and 4 articles respectively; in No. 10 leg I had 4 articles cut off. These ticks in due course moulted and gave rise to three females.

All of the previously amputated limbs were regenerated in the adults, only in No. 9 were legs II and III (those mutilated) slightly smaller than normal.

3. Hyalomma aegyptium.*Operations on the nymph and their effect on the adult.*

The legs were amputated in seven nymphs (Nos. 11-17) as follows:

<i>Hyalomma</i> No.	Number of articles amputated from legs	Regeneration in adult
11	2 from leg III	perfect
12	2 from leg IV	"
13	4 from legs II and III	„ (untouched opposite leg III small)
14	4 from leg I	„
15	3 from leg II	regenerated, distal articles very slightly shorter than normal
16	4 from legs I and II	regenerated, slightly shorter than normal
17	3-4 from legs I, II, III, IV	regenerated, legs II, III, IV somewhat smaller than normal

SURVEY OF THE RESULTS OBTAINED.

All the immature ticks in these experiments were operated upon within 1-2 hours of their becoming fully gorged and abandoning the host. Extensive mutilations may cause death through excessive loss of coelomic fluid. Moderate mutilations are well borne by ticks, 78 out of 108 survived the operations herein described; the heaviest loss followed operations affecting the basis capituli. One third-stage nymph (*Argas* 73), in which the basis had been cut across midway in the second nymphal stage, survived for four years in the laboratory although it was unable to feed because of its mutilated mouthparts¹. Metamorphosis may be retarded or not according to the severity of the mutilation that has been inflicted.

REGENERATION OF MOUTHPARTS.***Argas persicus.***

Operations on *larvae* wherein the mouthparts are mutilated shortly after the larva has abandoned the host in a fully gorged condition, cause the various structures to be differently affected in the first-stage nymph: the *palps* are not regenerated but appear as stumps which but for their closed and rounded ends correspond mostly in structure to the parts that were left intact in the larva (Fig. 4 A-E); occasionally an additional article or two is

¹ See p. 24.

regenerated. The *hypostome*, when amputated at any point short of its base, is perfectly regenerated, but if cut at its base the regenerated hypostome may be slightly deformed (Fig. 3 A). It should be noted that slight asymmetry and irregularities of dentition occur in all stages normally. The *digits of the chelicerae* are usually deformed to a varying degree (Fig. 4 D, K, L), the greatest deformity or even their non-regeneration may be caused by cutting off any considerable length of the shaft (Tick 14, Fig. 4 M); slightly deformed digits may function well enough for purposes of feeding.

First-stage nymphs, when mutilated as described in the case of the larvae, yield second-stage nymphs whose structures are affected as follows: the *palps* may appear like the stumps above described, they may be partly regenerated (*Argas* 28, 33, 53, 59), they may be completely regenerated whilst appearing short but about as broad as normal (*Argas* 32, 43), or they may appear normal. The *hypostome* in most cases is perfectly regenerated, but at times it is emarginated distally, an appearance occasionally met in ticks that are presumably normal. The *digits of the chelicerae* may be regenerated perfectly, or they may be undersized or deformed even when a length of shaft only equal to one or two digit-lengths is removed at operation. Slightly deformed digits do not prevent the mutilated ticks from feeding normally.

The whole series of second-stage nymphs, when left unmolested after the first operation, and adequately fed, gave rise to normal adults (19 specimens) or third-stage nymphs (two specimens) at the next moult.

When the *basis capituli* was cut across in first-stage nymphs only four out of ten of the ticks survived, the operation being accompanied by a great loss of coelomic fluid. None of the survivors moulted normally. In one case the basis capituli was not regenerated (*Argas* 51), in only one case was there an abortive attempt to regenerate a palp (*Argas* 54, Fig. 3 B and B'); the hypostome was not regenerated, at most, in one case it was represented by a smooth stump (*Argas* 50); the sheaths of the chelicerae were regenerated, they terminated either with apparently normal digits (*Argas* 60), small or deformed digits (*Argas* 54, Fig. 3 B), or they were devoid of digits (*Argas* 50, 51), these differences depending no doubt upon the degree of mutilation to which the parts had been subjected according as they were more or less protruded when amputated.

Second-stage nymphs, when mutilated, yielded adults (three specimens) or third-stage nymphs (five specimens) at the succeeding moult. In these the *palps* were all perfectly regenerated, in one tick the palp had been amputated basally. The *hypostome*, cut across basally, was perfectly regenerated in five cases, it appeared emarginated and asymmetrical in two, and in only one case did it subsequently develop to a toothless spine (*Argas* 67, Fig. 4 H). The *digits of the chelicerae* were regenerated perfectly in all but one case (*Argas* 70) wherein the external article of the digits appeared somewhat small. Only one of these ticks refused to feed (*Argas* 67 above referred to) and those that emerged as third-stage nymphs were subsequently raised to perfect adults.

When the *basis capituli* was cut across in second-stage nymphs, as was done successfully in two instances, almost perfect regeneration followed in one case (*Argas* 72), whilst in the other, where the basis was cut across half-way, the palps only reappeared as short stumps, the hypostome was absent and the digits deformed.

The results obtained with *Argas*, in respect to the power possessed by various immature stages of regenerating their mouthparts, are best elucidated by the following summary relating to the milder operations described in the foregoing pages:

OPERATIONS ON LARVAE

		RESULT IN NEXT STAGE
Palps amputated	21	2 regenerated partly 19 not regenerated
Hypostomes amputated	16	16 regenerated 9 perfect 5 slightly deformed 2 deformed
Digits amputated	18	17 regenerated 5 slightly deformed 8 deformed 4 much deformed 1 <i>not</i> regenerated

OPERATIONS ON FIRST-STAGE NYMPHS

Palps amputated	13	11 regenerated 2 perfect 5 short or imperfect 4 partially regenerated 2 <i>not</i> regenerated
Hypostomes amputated	20	20 regenerated 15 perfect 5 slightly deformed
Digits amputated	17	17 regenerated 5 perfect 6 slightly deformed 6 deformed or small

OPERATIONS ON SECOND-STAGE NYMPHS

Palps amputated	15	15 regenerated 15 perfect
Hypostomes amputated	8	8 regenerated 5 perfect 2 slightly deformed 1 badly deformed
Digits amputated	11	11 regenerated 9 perfect 2 slightly deformed

It should be noted, at the outset, that operations on *larvae* which have dropped off in a replete state from the host yield, on the whole, unfavourable

results in respect to regeneration for the reasons specified on pp. 17 and 23. It is noticeable that after operations on larvae the hypostome is regenerated better than the digits, whilst mostly no attempt at regeneration takes place in the palps.

When the *first-stage nymph* has its mouthparts amputated, the hypostome is best regenerated, then follow the digits, whilst the palps are least well regenerated.

This order changes, however, when operations are made in the *second-stage nymphs*, for the succeeding stage (adult or third-stage nymph) shows regeneration to have taken place best in the palps and digits and least well in the hypostome. The cause of this phenomenon requires elucidation.

The foregoing tabular summary shows, moreover, that as the tick develops toward maturity, the power to regenerate the palps and digits increases, there being no distinct difference in respect to the hypostome, although the proportion of perfect to slightly imperfect hypostomes in second-stage nymphs after operation is highest, *i.e.* 15 : 20.

Amblyomma hebraeum.

Operations on *larvae*, consisting of basal amputations of palps and hypostome, were followed in the nymph by perfect regeneration in all cases (ten operations); amputations of digits and twice their length of cheliceral shaft had no effect, but in one case, where a longer piece of shaft was removed (*Amblyomma* 5), the shaft appeared shorter than normal in the nymph.

Operations on *nymphs*, consisting of basal amputations of palps and hypostome or of digits with four times their length of shaft, were followed by complete regeneration of all parts in the adult.

Hyalomma aegyptium.

Operations on *nymphs* (ten specimens) wherein the palps and hypostome were amputated at or near the base, were followed by perfect regeneration of these parts in the adult. Amputations of the digits and 2-3 times their length of cheliceral shaft, only resulted in deformity of the digit in one case (*Hyalomma* 3), whereas, when more of the shaft was removed (*Hyalomma* 8), the digits appeared badly deformed in the adult.

The results obtained after amputations of mouthparts in the Ixodid ticks are therefore in strong contrast to those in *Argas*. Regeneration takes place equally well after operations on larvae and nymphs. Perfect regeneration followed on almost all operations, *i.e.* 27 hypostome amputations, 23 palp amputations and 40 amputations of digits; slight deformity of digits followed three operations, great deformity in two, and in but one case was the cheliceral shaft shortened through mutilation.

REGENERATION OF LEGS.

Argas persicus. It was found that when freshly gorged larvae had their legs amputated, the corresponding limbs were not regenerated in the first nymphal stage, but when these nymphs were fed and allowed to undergo a further moult without operative interference, they regenerated these mutilated limbs perfectly. When first-stage nymphs, under like conditions, were similarly mutilated, they gave rise to second-stage nymphs with well-formed legs of subnormal size. These results are in conformity with those of Hindle and Cunliffe (cited on p. 7) who explain the difference in the results obtained with larvae and nymphs by an interesting observation on larvae subjected to operations made at an earlier stage whilst still feeding on the host. When replete larvae abandon the host, they are well advanced in their nymphal development, and, consequently, if they are operated upon at this stage, they do not regenerate their legs, the first-stage nymphal formative tissues having been injured.

Amblyomma hebraeum. My experiments on this species show that when the legs are mutilated in replete larvae or nymphs immediately after the ticks have abandoned the host, the limbs are regenerated in the succeeding stage; in one adult that developed from a nymph which had been mutilated, the regenerated limbs were slightly smaller than normal, in the remaining six ticks the regenerated limbs were of normal size.

Hyalomma aegyptium. Similar experiments of mine upon replete nymphs of this species show that the regenerated limbs in the adult may be either perfect or of subnormal size.

Therefore 21 leg amputations in Ixodid ticks were followed in all cases by regeneration, 13 legs being normal and 8 slightly reduced in size.

SUMMARY AND CONCLUSIONS.

Amputation experiments upon immature stages of *Argas persicus* (Oken 1818), *Amblyomma hebraeum* Koch 1844, and *Hyalomma aegyptium* (Linnaeus 1746) show that the mouthparts and legs of these ticks may be more or less regenerated when mutilated shortly after the ticks have abandoned the host in a fully engorged condition.

Regeneration of Mouthparts.

In immature *Argas persicus* the mouthparts are regenerated more or less perfectly according to the structure affected and the stage of development of the tick. Freshly gorged larvae regenerate their mouthparts badly compared to first and second-stage nymphs because during their period of parasitism upon the host (5-6 days usually) the larvae have advanced well along the road to becoming nymphs, therefore the operation largely affects the formative tissues of the developing nymph. The nymphs feed rapidly, usually in

15–20 minutes, and proceed with their metamorphosis after abandoning the host, consequently, when they are mutilated soon after feeding, they regenerate their mouthparts (and legs) better than do the larvae. After operations on larvae and first-stage nymphs regeneration takes place best in the hypostome, next in the chelicerae and least well in the palps. This order was reversed after similar operations on second-stage nymphs. The power to regenerate palps and chelicerae grows as the tick approaches maturity, but it remains fairly constant for the hypostome. Amputations made through the basis capituli usually render the tick incapable of feeding after it has moulted to the next stage, the mouthparts not having been adequately regenerated. In one case (*Argas* 72), however, the amputation of the anterior part of the capitulum with its appendages in a second-stage nymph was followed by almost perfect regeneration of the part in the succeeding third-stage nymph. Where regeneration did not take place adequately after severer operations on the basis capituli, as exemplified in another case (*Argas* 73), the mutilated tick lived on without its capitulum for four years without a meal. This experiment explains the origin of the anomalous “headless female” of *Ixodes ricinus*, referred to and figured by Wheeler¹, which lived four years and was lost. Lesser mutilations, *i.e.* those affecting the appendages only, are frequently followed by perfect regeneration if the injury is slight. Imperfectly regenerated parts are often capable of functioning so that the tick can feed. Such a tick, if not interfered with, will acquire perfectly normal mouthparts at the next moult.

The immature stages of the Ixodid ticks *A. hebraeum* and *H. aegyptium* behave differently to *Argas persicus* when injured soon after abandoning the host, in that they possess much greater powers of regeneration. Basal or partial amputations of the hypostome and palps, or moderate mutilations of the chelicerae, are followed by perfect regeneration in most cases.

The greater power of regeneration possessed by Ixodid ticks bears directly upon their parasitic habits. The slowly feeding larval stages of Argasids (*A. persicus*, *A. vespertilionis*, for example) possess relatively more dentate hypostomes than do the adults which are rapid feeders. With the exception of *Haemaphysalis concinna* (vide Brumpt's observations recorded by me in *Parasitology*, VII. 434) all known Ixodids are slow feeders and an examination of genera like *Ixodes*, *Amblyomma* and *Hyalomma* proves that their hypostomes are most efficient organs for anchoring the tick to the host. The accompanying illustrations (Figs. 5 and 6) of the capitulum of *A. hebraeum* and *H. aegyptium* females show that they possess long, highly dentate hypostomes broadening distally and liable to break where they narrow toward the base when violence is done to the tick that is fixed thereby to the skin. The examination of such Ixodid ticks collected in the field proves how frequently their long hypostomes get broken off near the base, and consequently how vital it is that they should be readily regenerated. Whilst a tick may save

¹ Wheeler, E. G. (iii. 1906). British Ticks. *Journ. of Agricult. Sci.* I. p. 401, Plate X, fig. 38.

its digits by moving and retracting them, it cannot do so with its hypostome which is a rigid structure whose chitin is continuous with that forming the basis capituli. The hypostome is the most readily regenerated element of the mouthparts because it consists almost entirely of chitin, its formative tissues lying beneath the stout chitinous base whilst it is developing in the

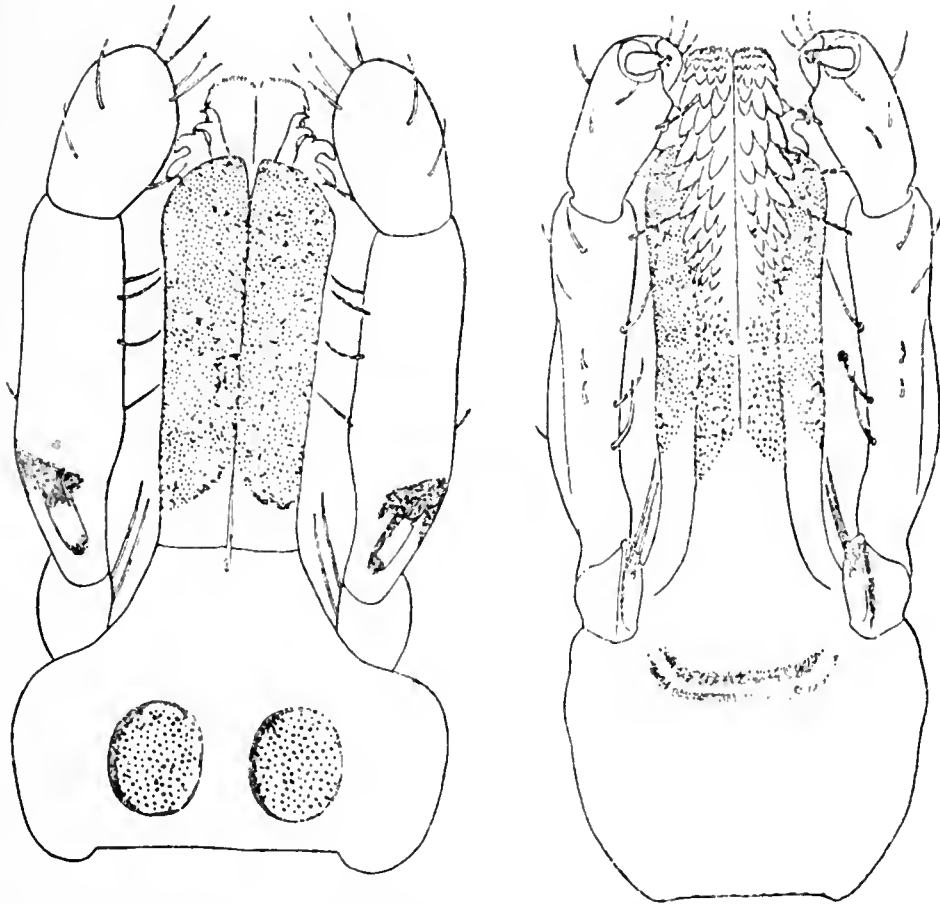


Fig. 5. *Amblyomma hebraeum* ♀. Capitulum in dorsal and ventral aspect (Original, ca. $\times 16$).

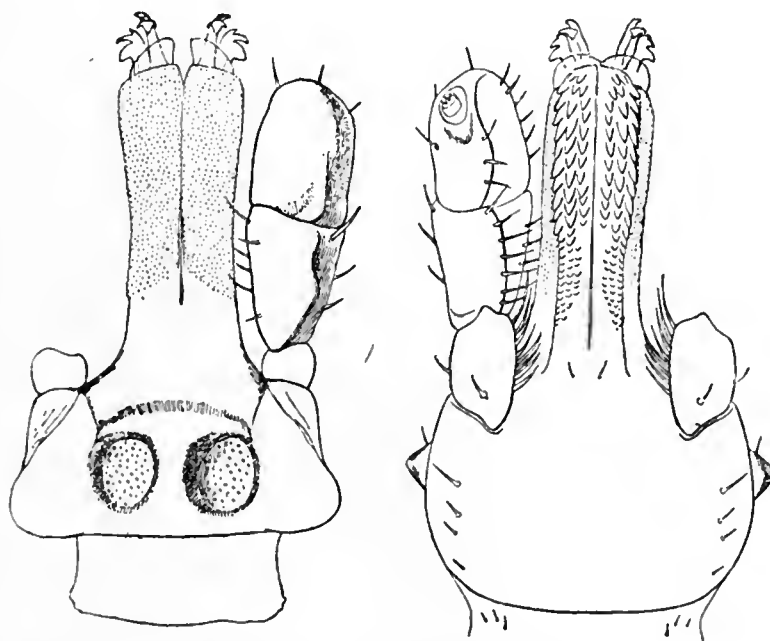


Fig. 6. *Hyalomma aegyptium* ♀. Capitulum in dorsal and ventral aspect (Nuttall, 1911 ca. $\times 16$).

immature stages prior to a moult. In this position, the formative tissues are not liable to be hurt, whereas in the case of the palps and chelicerae as with the legs, the formative tissues are more directly accessible to injury.

The power of regenerating mutilated mouthparts possessed by immature ticks is of paramount importance in connection with their maintenance in

nature where they are frequently injured through their forcible removal from the host, the hooked hypostome and digits of the chelicerae being broken off to a varying degree because they are so firmly anchored in the host's skin. Such mutilation of the mouthparts is much more likely to occur in Ixodid than in Argasid ticks because the mouthparts of the latter are as a rule less effective anchoring organs, the structure of the mouthparts in the two groups being correlated with their feeding habits upon the host.

Regeneration of legs.

The experiments with *Argas persicus* herein recorded confirm those of Hindle and Cunliffe (*loc. cit.*). If the legs of the larva are amputated shortly after the tick has abandoned the host in a fully gorged condition, the first-stage nymph usually shows stumps corresponding in length to the portion of limb that was left intact in the larva. The authors cited found, however, that if the larva had its legs amputated whilst attached and feeding on the host, *i.e.* 2-3 days prior to its dropping off in a replete state, that the legs may at times be regenerated in which case they are usually of subnormal size. When the legs of first and second-stage nymphs are amputated soon after they have fed, the limbs are regenerated but are usually small. Immature ticks with small or stumpy legs, if not subjected to further interference, develop normal limbs after a further moult.

My experiments with *A. hebraeum* and *H. aegyptium* show that these Ixodid ticks possess greater powers of regeneration than *A. persicus* in respect to the legs, this being in harmony with the results above described in connection with the mouthparts. The number of legs amputated from the two Ixodid species in the larval and nymphal stages was 21 and all of them were regenerated; 13 limbs were of normal size and 8 slightly smaller than normal.

SCLEROSTOMES OF THE DONKEY IN ZANZIBAR AND EAST AFRICA.

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(With 5 Text-figures.)

INTRODUCTION.

DURING the last few years our knowledge of the Sclerostome parasites of the horse has been greatly augmented, the nematodes of the donkey have not however received attention since 1901, the date of publication of Looss' famous monograph on the Sclerostomes of the Equidae.

I have recently received for study two collections of helminths from domestic animals, each of which includes a number of nematodes from the donkey; for the first of these I am indebted to Mr W. M. Aders, Economic Biologist of the Zanzibar Government, the second collection was kindly entrusted to me by Professor G. H. F. Nuttall, F.R.S., who had received it from Mr R. Eustace Montgomery of the Veterinary Pathological Laboratory, Nairobi.

Some of the material from the latter source reached me in a poor state of preservation and it was not possible to identify all the forms present, altogether however nine species were observed, including two hitherto undescribed:

1. *Strongylus vulgaris* (Looss).
2. *Strongylus edentatus* (Looss).
3. *Strongylus asini* sp. n.
4. *Triodontophorus intermedius* Sweet.
5. *Cylicostomum auriculatum* (Looss).
6. *Cylicostomum coronatum* (Looss).
7. *Cylicostomum bicoronatum* (Looss).
8. *Cylicostomum alveatum* (Looss).
9. *Cylicostomum adersi* sp. n.

The worms numbered 1-3 occurred in both collections, 4-8 were from East Africa, whilst No. 9 was from Zanzibar only.

Since the publication of Looss' monograph a number of new species have been referred to the genera *Triodontophorus*, *Gyalocephalus* and *Cylicostomum*,

and other additions will no doubt be made, especially to the last-named genus, as more material from horses and donkeys is investigated; it is however interesting to find an undescribed species among the larger *Sclerostomes* belonging to the genus *Strongylus* s.s.

The new species from the donkey is a large form, in some respects intermediate between *Strongylus equinus* (Looss) and *S. edentatus* (Looss), but possessing some characteristic features peculiar to the species, it will no doubt be found not to be restricted to that host but to occur in the horse as well.

Owing to the thickness of the head and neck muscles in the larger forms of *Strongylus* I have found the anatomy of these worms somewhat difficult to make out in glycerine mounts and I have found it desirable to examine them in a medium of rather higher refractive index. For this purpose pure white creasote (into which the worms may be transferred direct from alcohol without shrinkage) has proved very suitable. I have to thank Mr H. A. Baylis, of the British Museum, for calling my attention to this very useful reagent.

Genus *STRONGYLUS* Mueller

(= *Sclerostoma* Rudolphi).

***Strongylus asini* sp. n.**

SPECIFIC DIAGNOSIS. *Strongylus*: Body large, 18–42 mm. in length with a maximum thickness of 1.8–2.5 mm.

The head (0.6–1.3 mm. in breadth) is divided from the rest of the body by a slight constriction to form a neck-like region, similar to, but not nearly so well defined as that of *S. edentatus*.

The mouth is circular and of considerable size (320–470 μ in diameter). The mouth collar is deep and sharply marked off from the rest of the skin.

External and internal leaf-crowns are of the type usual in *Strongylus*, especially resembling those of *S. equinus*. The lateral and submedian head-papillae are also as in the other species of the genus.

The mouth capsule is strongly developed, like that of *S. edentatus* it appears markedly cup-shaped when seen in a dorsal or ventral view (Fig. 1 A), in a lateral view (Fig. 1 B) the dorsal wall is seen to be shorter and more convex than the ventral, thus recalling the similar structure of *S. vulgaris*.

The dorsal gutter is well developed but considerably shorter than in the other species, its anterior termination being some distance behind the anterior margin of the mouth capsule (Fig. 1). A single tooth arises from the base of the dorsal gutter, like that of *S. vulgaris* it is divided into two broad lateral projections which are however relatively much lower than in that species and subdivided by ill-defined grooves into a number of rounded cusps. The latter are variable in number, in the majority of specimens, however, three principal cusps are conspicuous (Fig. 1 A), each composed of several smaller ones. There are no ventral teeth such as are found in *S. equinus*.

The excretory pore is situated in the head region as in *S. equinus* and *S. edentatus*.

Oesophagus: 1.6–2.4 mm. in length. The cervical papillae are in the neighbourhood of the nerve-ring, about 1.5 mm. from the anterior extremity of the body.

Female: 30–42 mm. in length, 1.8–2.5 mm. in extreme breadth. The head has a width of 0.9–1.3 mm.

Vulva 6–7 mm. from the posterior end of the body. The tail region is similar to that of *S. equinus* and *S. edentatus*; the anus is 0.4–0.6 mm. from the posterior extremity.

Male: 18–32 mm. in length, with a maximum breadth of about 1.8 mm. The head is 0.6–0.9 mm. broad.

As in the two larger of the known species of *Strongylus* the genital bursa is small as compared with the size of the body, it measures 1.1 in width in the largest specimen before me. The median lobe is short (Fig. 2).

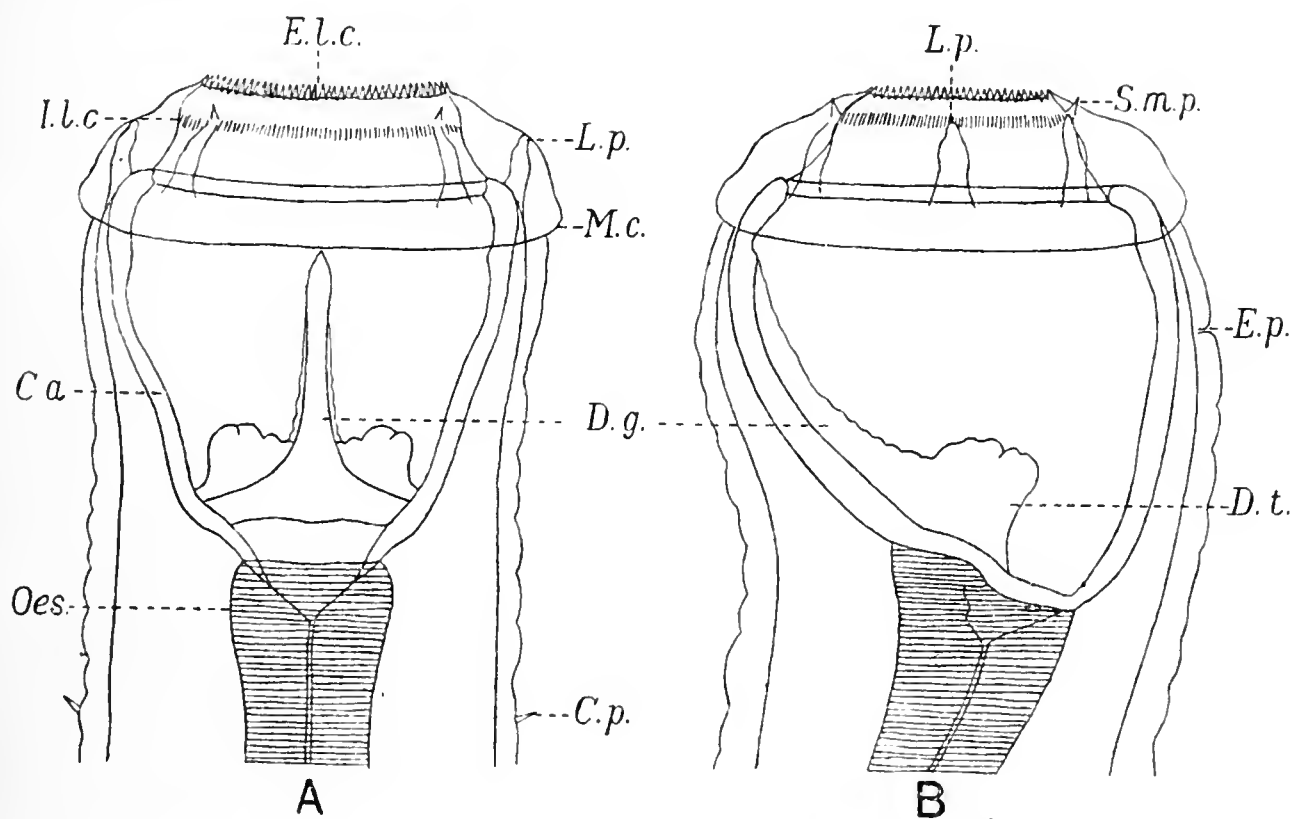


Fig. 1. *Strongylus asini* sp. n. A. Dorsal view of anterior extremity, $\times 37$. B. Lateral view of anterior extremity of another specimen, $\times 37$.

The rays of the bursa are similar to those of *S. edentatus*, *i.e.* they are more slender than those of *S. equinus*. The branches of the posterior rays differ from those of the other species in that the inner (dorsal) branch is simple, the external branch divided, *i.e.* the converse of the arrangement prevailing in the genus. This character may however not be specific. I was able to examine three male specimens only and the mode of branching of the posterior rays is liable to variation in many species of Strongyles. The spicules measure approximately 1.6 mm. in length.

Habitat: (1) Zanzibar, in a cyst of the liver and in the caecum of the donkey. (2) Nairobi, East Africa, in the caecum of the donkey.

The Zanzibar specimens consisted of three females and one male (all fully developed) from the liver cyst, whilst another male was found among a number of specimens of *S. edentatus* and *S. vulgaris* from the caecum. The Nairobi

collection contained one male in a good state of preservation, whilst a number of others occurred in material which had evidently been dry at some period, the worms being greatly shrivelled and full of air-bubbles; the species of *Strongylus* could only be identified by dissecting out the mouth capsule after maceration with Eau de Javelle. The specific diagnosis is therefore based on six specimens only (3 ♂♂ and 3 ♀♀).

The occurrence of fully developed specimens of this form in the liver is a point of interest, immature specimens of *S. equinus* have been observed in this situation but are more commonly met with in the pancreas; stages of this species and of *S. edentatus* have also been found in the hepatic ligament (Railliet and Henry 1902, Railliet 1915).

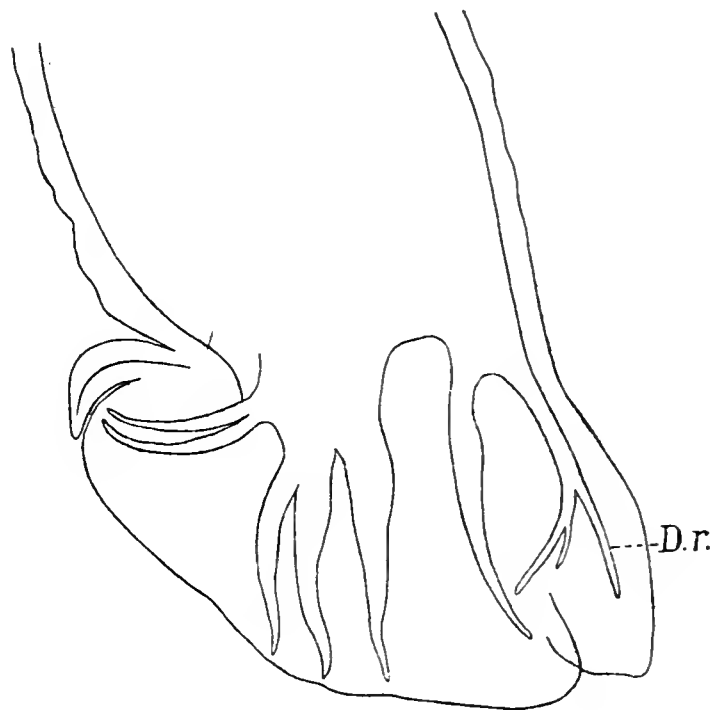


Fig. 2. *Strongylus asini* sp. n. Lateral view of male bursa, $\times 37$.

Genus CYLICOSTOMUM Railliet and Henry¹

(= *Cylichnostomum* Looss).

Cylicostomum adersi sp. n.

A single male specimen of this worm was found in Mr Aders' collection; the specific diagnosis is therefore based on this sex only.

SPECIFIC DIAGNOSIS. *Cylicostomum*: A large species presenting much the same appearance as *C. insigne* Boulenger (1917).

Head marked off from the body by a slight neck-like constriction. The mouth collar is comparatively narrow (Fig. 3).

The lateral head-papillae do not project from the surface of the collar, submedian papillae short and leaf-shaped.

The external leaf-crown consists of about 26 large pointed leaves surrounding the circular mouth-opening, the internal leaf-crown of double this number of similar but less conspicuous elements.

¹ In a recent paper (1917) I used Looss' generic name for these worms, there seems however no doubt that the form *Cylicostomum* has priority.

The mouth capsule is broad, having a depth of 70μ with a breadth of 140μ . The wall of the capsule is increased posteriorly to form a very well-marked "hoop-like" thickening (Fig. 3).

A dorsal gutter is present but is very short, just projecting into the mouth capsule. There is a well-developed oesophageal funnel (Fig. 3).

Male: 14 mm. in length, with a maximum breadth about .75 mm. near the middle of the body. The head is nearly 200μ broad.

The oesophagus has a length of 700μ ; it reaches its maximum breadth

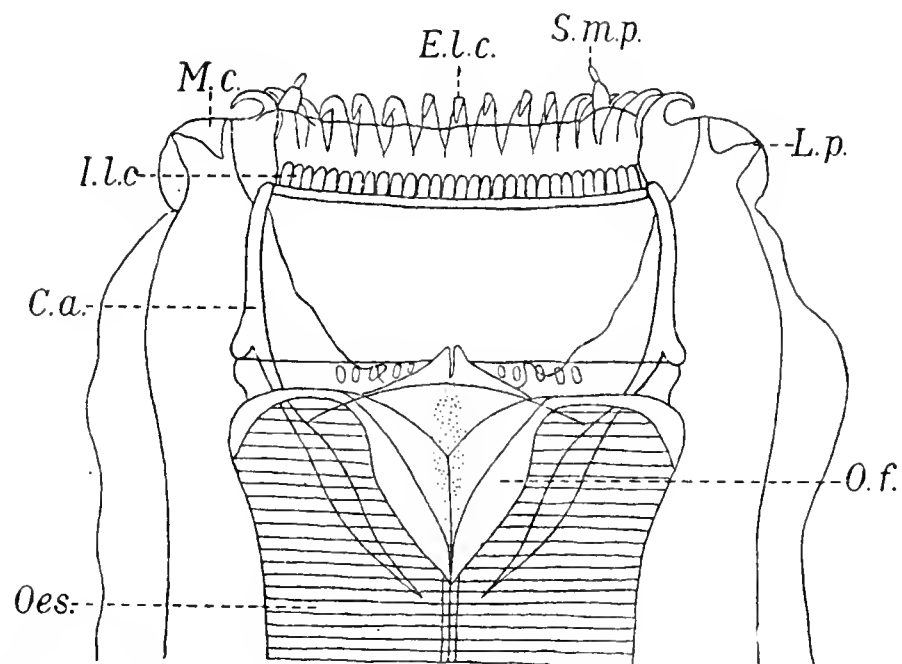


Fig. 3. *Cylicostomum adersi* sp. n. Anterior extremity in dorsal view, $\times 110$.

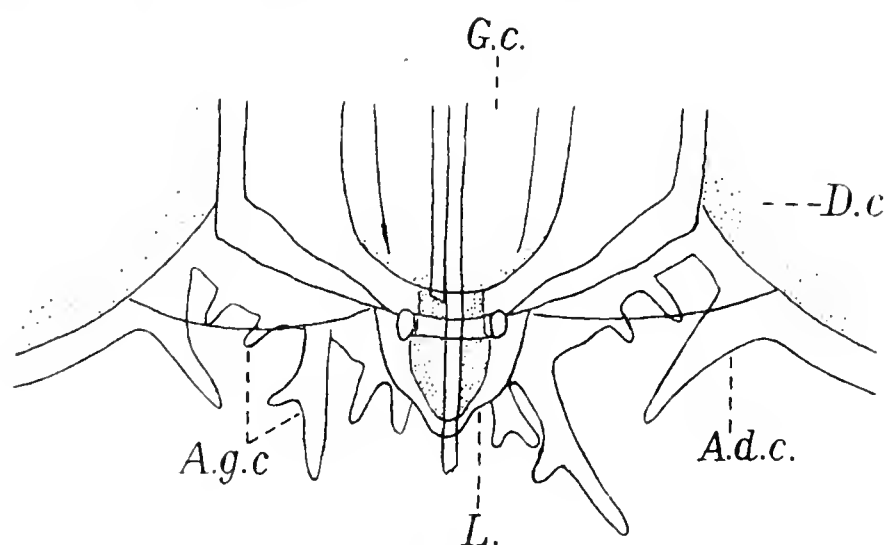


Fig. 4. *Cylicostomum adersi* sp. n. Ventral view of the genital cone to show the appendages, $\times 280$.

(170μ) about the middle of its posterior half. Cervical papillae are situated 650μ from the anterior extremity of the body.

The bursa (Fig. 5) has a broad median lobe of moderate length. The comparatively short genital cone is completely surrounded by a well-developed dermal collar. Prebursal papillae slender and very long (250μ).

The appendages of the genital cone are completely fused in the middle line, forming a thin semicircular plate the margin of which bears four pairs of delicate finger-shaped processes, some of which have bifurcated extremities. On each side of this plate is an additional process arising from the dermal collar (Fig. 4).

Habitat: Zanzibar, caecum of donkey.

This species is evidently closely allied to *C. insigne* and belongs to the group of species which includes this form and *C. elongatum*, *C. radiatum* and *C. auriculatum*. It is distinguishable from *C. insigne* by the character of the genital appendages, by the number and shape of the leaves of the internal leaf-crown, and the presence of a highly chitinized oesophageal funnel.

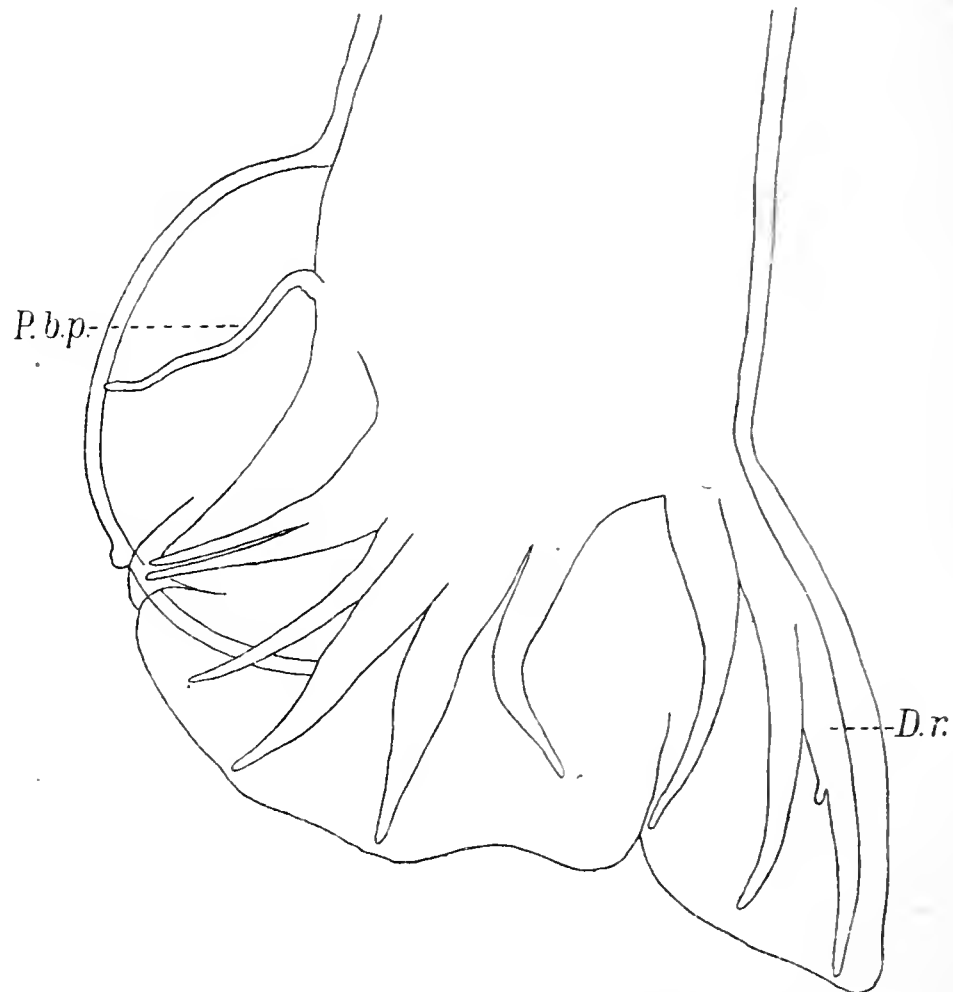


Fig. 5. *Cylicostomum adersi* sp. n. Lateral view of male bursa, $\times 75$.

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EXPLANATION OF LETTERING.

<i>A.d.c.</i> Appendage of the dermal collar.	<i>G.c.</i> Genital cone.
<i>A.g.c.</i> Appendage of the genital cone.	<i>I.l.c.</i> Internal leaf-crown.
<i>Ca.</i> Wall of mouth capsule.	<i>L.</i> Ventral lip of the genital cone.
<i>C.p.</i> Cervical papilla.	<i>L.p.</i> Lateral head-papilla.
<i>D.c.</i> Dermal collar of the genital cone	<i>M.c.</i> Mouth collar.
<i>D.g.</i> Dorsal gutter.	<i>Oes.</i> Oesophagus.
<i>D.r.</i> Dorsal ray of the bursa.	<i>O.f.</i> Oesophageal funnel.
<i>D.t.</i> Dorsal tooth.	<i>P.b.p.</i> Prebursal papilla.
<i>E.p.</i> Excretory pore.	<i>Sp.</i> Spicule.
<i>E.l.c.</i> External leaf-crown.	<i>S.m.p.</i> Submedian head-papilla.

OBSERVATIONS ON BILHARZIASIS AMONGST THE EGYPTIAN EXPEDITIONARY FORCE.

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(With Plates III-V and 3 Text-figures.)

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I. HISTORICAL.

IN 1851, Bilharz (1852) first discovered paired adult trematode worms originally named after him in the portal system of an Egyptian fellah. Subsequently, investigations of the deposits of ova in excreta were made, but numerous attempts by Cobbold, Sonsino, Lortet and Vialleton (1894) and others, to unravel the life history of the parasite, failed. Looss (1896), appreciating that the digenetic trematodes must necessarily pass through a molluscan intermediary, dissected many species of snails collected from the fresh-water canals around Cairo. He failed to find the cercariae of bilharzia, and discarded the hypothesis of an intermediate molluscan host. As, however, the miracidium of *Schistosomum*

haematobium contained germinal cells within its body cavity, it must, naturally, be argued, have been destined to produce sporocysts at some stage of its life cycle. In view of this, Looss evolved the hypothesis that man acted simultaneously as the intermediary, as well as the definitive host of this parasite.

According to his hypothesis the miracidia after reaching water perforated directly the skin of man, thence making their way to the liver. Here they developed into male and female sporocysts which in turn gave rise to male and female cercariae, and these, in their turn, developed into adult worms. Manson on the basis of biological analogy always opposed this view.

Looss also denied the existence of two species of bilharzial parasites, basing his contention on the absence of constant morphological differences in the two hypothetical species. He regarded the lateral spined ovum as characteristic of the unfertilized, and the terminal spined ovum as the one associated with the fertilized female; further he maintained that he had seen both varieties of ova simultaneously in the uterus of the same female worm.

Sambon (1907) supporting the views of Manson (1903), and Sonsino, wrote an able paper establishing the identity of a new species of bilharzia which he named *Schistosomum mansoni*. He based his reasoning on the different geographical distribution of the two forms of infestation, on the different systems pathologically involved, and on the morphological differences in the ova of the two species. Looss bitterly opposed his reasoning.

Bour (1913) failed to convey the disease to monkeys by injecting miracidia intravenously.

Miyagawa (1912) described the entrance of the infesting form of *S. japonicum*, an allied species, through the skin of the host, to the portal system. Miyairi and Suzuki (1913) demonstrated that the fresh-water mollusc, since named *Blandfordia nosophora*, acted as the intermediate host of *Schistosomum japonicum*. These investigators also conveyed the disease to mice by placing them in water containing infested snails. Ogata (1914) accurately described the morphology of the cercaria of *S. japonicum*, and later, Leiper and Atkinson (1915) confirmed all these observations, in Japan. In 1915, Leiper came in charge of the Bilharzia Mission to Egypt. As a result of the work of this mission, the existence was established of two species of *Schistosoma* morphologically distinct and producing different ova. From these ova, miracidia are hatched, which developing in certain fresh-water molluscs, produce cercariae, which have slight morphological distinctions.

The transmission of the disease to laboratory animals was effected and the affinity of *S. haematobium* for the vesical, and *S. mansoni* for the alimentary systems demonstrated.

In 1915-16, Cawston, in South Africa, showed that a fresh-water mollusc *Physopsis africana* (a near relative of *Bullinus*) acts as the intermediary host of *S. haematobium* in that country, and, together with Becker, claims to have produced artificial infestation of this species of snail with miracidia

hatched from terminal spined ova, and has described the morphology of the cercariae so produced.

Lutz (1917) and Iturbe (1917) have confirmed Leiper's observations in Brazil and Venezuela respectively by tracing the development of *Schistosomum mansoni* in the local species of *Planorbis* (*P. olivaceus*), and the former has succeeded in infesting rabbits and guinea-pigs under experimental conditions.

It is only fair to state that after the Japanese discoveries were published, Looss, still mindful of his own failure and that of Sonsino to identify bilharzia cercariae amongst the numerous developmental forms found in snails in the endemic centres of Egypt, suspected that *Schistosomum haematobium* had a life history which materially differed from *S. japonicum*.

In estimating Leiper's work on this subject one should not forget that was the prevalent view at the beginning of the war, and furthermore the real value of much of the Japanese work was lost to science at that time because the original articles were printed in Japanese journals in the native language and had not then been translated into either German or English.

II. SCOPE OF THE PRESENT INVESTIGATION.

The experimental data and observations on which this paper is based were collected after the departure of the Bilharzia Mission from Egypt at different periods, as far as military exigencies would permit, from 1916 onwards till the end of 1918. Through the generosity of the Australian Red Cross Society and of the Australian Military authorities, the cost of the experimental monkeys which were utilized was defrayed.

During these years a large number of troops, Imperial, Colonial and Indian, were stationed in districts heavily infested with bilharziasis. Although timely warnings were given by Dr Leiper and his Commission, a considerable number of troops became infested. From the fact that isolated posts were maintained during 1916 and part of 1917 along the Sweet-water Canal, and large bodies of troops were stationed in the Fayoum in close proximity to various collections of water it is not surprising that many men became victims of the disease.

It is probable that we have been able to trace but a small percentage of the infections originally acquired in Egypt, as a large number of men who had been stationed in the endemic zones were moved to France during the years 1916 and 1917 and probably only developed the symptoms of the disease at a later period.

The earliest cases among the Australian troops were accompanied by pyrexia, urticaria and general toxæmia and thus escaped recognition at first sight. These were probably examples of a massive infection; in the later cases, especially the more chronic vesical infections, these generalized symptoms were not so common.

The results of the present enquiry, which has entailed a considerable amount of work and the dissection of over 10,000 snails, has been to confirm

in a remarkable manner the historic investigations of Leiper, Thompson and Cockin, both as regards the duality of the species of Schistosomidae, and as regards their selective affinity for the two genera of fresh-water molluscs.

The data which have been obtained from the accidental infestation of the troops in different parts of Egypt in association with the local snail fauna amply confirm those obtained from experimental animals.

In addition, certain observations were made on the distribution of the different species of snails and their habits which have a bearing upon the prophylaxis of the disease, and it is with this end in view, and also with the idea of promulgating further investigations on the general prophylaxis of bilharziasis, that we submit this paper for publication.

III. THE MORPHOLOGY AND LIFE CYCLE OF THE TWO SPECIES OF EGYPTIAN SCHISTOSOMIDAE.

(*Schistosomum haematobium*—Bilharz v. Siebold, 1852.)

(*Schistosomum mansoni*—Sambon, 1907.)

In *Schistosomum haematobium* infestations terminal spined ova are deposited in the excreta, both urine and faeces, of an infested subject. On reaching water a ciliated miracidium is hatched out, which in a period of thirty-six hours at a maximum must find the correct species of snail of the genus *Bullinus*, failing which it dies. If a suitable snail is encountered, the miracidium enters it by piercing its soft parts, probably through the pulmonary chamber, and eventually reaches the digestive and the hermaphrodite glands. Here it is converted into a morula and later becomes hollowed out into elongated finger-like sporocysts; these, by a process of budding of cell masses known as "germ balls" or blastospheres, produce cercariae; they may also by a process of exogenous budding produce secondary cysts. When mature, the cercariae are ejected into water, and in the subsequent twenty-four hours must meet their definitive host, usually man, and after invading his tissues, either viâ the skin or the upper alimentary tract, make their way to the portal veins in which they develop into adult worms, both male and female.

In *Schistosomum mansoni* infestations lateral spined ova are excreted in the faeces of an infested subject, less commonly in the urine as well. The intermediate host in this case also is a fresh-water snail, but of the genus *Planorbis*. In other respects the life cycle resembles that described above for *S. haematobium*.

THE OVA.

The ova of the various species of schistosomes affecting man are passed to the exterior in the excreta, and in this fashion reach water. These ova are of a dark yellow brown colour, and of different shape; they are non-operculated, but possess either a spine or the rudiments of one. The ovum is covered by a chitinous shell, lined with a thin shell membrane, its interior

being practically filled by a fully developed ciliated miracidium (Plate III, figs. 1 and 2).

The following table, Table I, will serve to illustrate the main difference in the ova of the three species of *Schistosoma*.

Table I.

Differences in the Ova of the various Schistosomidae affecting man.

<i>Schistosomum japonicum</i> (Katsurada, 1904) vel. <i>S. cattoi</i> (Blanchard)	<i>Schistosomum mansoni</i> (Sambon, 1907)	<i>Schistosomum haematobium</i> (Billh. v. Sieb. 1852)
(1) <i>Ovum</i> 70–75 μ long, 45–55 μ broad	<i>Ovum</i> closely resembles <i>S. haematobium</i> , but is on the average slightly shorter	<i>Ovum</i> 120–160 μ long, 40–60 μ wide
(2) <i>Shape</i> more rounded than other species	<i>Shape</i> Oval or spindle-shaped	<i>Shape</i> Oval or spindle- shaped
(3) <i>Spine</i> . No spine but a rudimentary lateral papilla	<i>Spine</i> . Lateral spine	<i>Spine</i> . Terminal spine
(4) <i>Excreted</i> in faeces, not in urine	<i>Excreted</i> in faeces, less frequently in urine	<i>Excreted</i> in urine and faeces

HATCHING OF THE OVA.

The ova may be readily hatched out by lowering the salt content of the fluid which bathes them, this occurs best at a temperature of 100° F. Microscopically, the first change noted under these conditions is a movement in the lateral walls of the ovum, at the point of its greatest transverse diameter. Activity of the cilia covering the miracidium is then noted and fluid currents appear to be set up within the shell membrane. This actual process, we believe, is due to the chitinous envelope and shell membrane acting conjointly as a semipermeable membrane, with the resultant passage of water from the fluid of lower to one of higher osmotic pressure. This ingress of water swells out the spindle-shaped ovum and increases its transverse diameter, and the miracidium, stimulated by the change in its environment, starts its ciliated activity. These two factors result in rupture of the chitinous shell in a lateral direction, as a general rule, slightly posterior to its central transverse axis.

Occasionally free miracidia may be found in freshly voided urine or faeces, but such an occurrence is very exceptional. After rupture of the chitinous envelope the miracidium still enveloped in the shell membrane may be seen partially extruded through the opening thus produced. Rupture of the membrane generally occurs during this process of extrusion, and is probably due to its tearing on the jagged chitinous edges. Once freedom is attained the miracidium swims rapidly away on its adventurous quest.

MIRACIDIA.

A limit of 36 hours is set down in which the miracidium must meet its specific intermediate host. In structure it closely resembles the miracidium of *Fasciola hepatica*, the most marked difference being the absence of eye spots.

It is covered with cilia, except at the anterior end where a papilla is situated. Excretory organs, and round clear germinal cells are both noticeable in the body cavity, and two or three transverse constrictions may be noted (while the miracidium is still unhatched) as grooves or indentations of the lateral borders (Plate I, figs. 1 and 2). On reaching its specific intermediate host, the previously enumerated species of *Bullinus* snail in the case of *Schistosomum haematobium*, and in the case of *Schistosomum mansoni*, *Planorbis boissyi*, the miracidium gains entrance by boring through the soft tissues, and eventually reaches the digestive gland, or liver.

Here, as previously noted, by proliferation of its germinal cells, the mother and daughter sporocysts are formed, and the ultimate development of the cercariae takes place in their interior.

A sporocyst is a simple sac, without either alimentary canal or oral sucker. The sporocysts under consideration are elongated finger-like cysts with thin walls, thickened at either end. They have independent but feeble power of movement, and appear to absorb nourishment directly through their walls. Over distension of the daughter cysts leads to rupture, and the evacuation of their contents. Where rupture takes place prematurely, we noted the following developmental forms:

- (1) Rounded or oval masses of cells.
- (2) Elongated oval masses of cells showing a tendency to form a posterior process.
- (3) A shapeless type of cercaria, with a partially developed anterior part, and a blunt posterior part—the future tail. The earliest indication of the forked nature of the latter is seen in two small buds at its posterior end.
- (4) The fully developed mature cercaria with anterior and lateral suckers, and a long bifid tail.

The above enumerated forms evidently represent the different stages in the development of the cercaria from the germinal cells of the sporocyst. The enormous development which may take place is shown in Plate V, fig. 1, where almost all the normal glandular tissue is displaced by sporocysts.

MORPHOLOGY OF CERCARIAE OF *S. HAEMATOBIIUM* AND *S. MANSONI*.

The specimens for this purpose were obtained by dissection of the livers of infected snails of the species, *Planorbis boissyi*, and *Bullinus contortus* and *dybowskii*.

These organs were teased out and the contained cercariae killed by heat. The specimens were then examined under a cover-glass. No marked difference was noted in their internal structure from the description given by Leiper in his original paper on the subject and the more recent minute one by Faust (1919). In live specimens the movements also correspond. In the main points of structure they resemble the cercaria of *S. japonicum*, as described by Ogata (*Verh. der Japan. path. Gesellsch.* Tokyo, 1914, vol. 48).

All *Schistosoma cercariae*, whether of the species *S. haematobium*, *S. mansoni*, or *S. japonicum*, belong to the furcocercous group of the Distome cercariae: Leiper emphasizes the following common characteristics of the Bilharzia group of cercariae.

- (1) The absence of the pharynx.
- (2) The presence of a forked or bifid tail.
- (3) The presence of anterior and ventral suckers.
- (4) The absence of eye spots.
- (5) The presence of two or more sets of glands situated posteriorly, one on each side of the body, and communicating with the mouth.

The following measurements for the cercariae of *S. mansoni* were made from 40, and those of *S. haematobium* from 35, freshly dissected specimens. The measurements are as tabulated in Table II below.

Table II.

Summary of Measurements.

Column I Cercariae of <i>S. japonicum</i> (Leiper)	Column II Cercariae of <i>S. mansoni</i>	Column III Cercariae of <i>S. haematobium</i>
1. Total length of <i>body</i> and <i>tail</i> 0.25 mm.	Total length of <i>body</i> and <i>tail</i> shorter than <i>haematobium</i> 0.374 mm.	Total length of <i>body</i> and <i>tail</i> , longer 0.398 mm.
2. <i>Body</i> 0.1 mm.	<i>Body</i> , slightly shorter 0.161 mm.	<i>Body</i> , slightly longer 0.190 mm.
3. Breadth of <i>body</i> 0.04 mm.	Breadth of <i>body</i> 0.060 mm.	Breadth of <i>body</i> 0.064 mm.
4. Post prolongation of <i>oral sucker</i> from anterior extremity 0.04 mm.	Posterior prolongation of <i>oral sucker</i> , from anterior extremity 0.053 mm.	Posterior prolongation of <i>oral sucker</i> , from anterior extremity 0.073 mm.
5. Ventral sucker	<i>Ventral sucker</i> , from posterior extremity 0.044 mm.	<i>Ventral sucker</i> , from posterior extremity 0.049 mm.
6. Length of <i>tail</i> to <i>bifurcation</i> 0.1 mm.	Length of <i>tail</i> to <i>bifur-</i> <i>cation</i> , longer 0.213 mm.	Length of <i>tail</i> to <i>bifur-</i> <i>cation</i> , shorter 0.208 mm.
7. Breadth of <i>tail</i> 0.01 mm.	Breadth of <i>tail</i> 0.035 mm.	Breadth of <i>tail</i> 0.031 mm.
8. Length of each arm of fork 0.005 mm.	Length of <i>each arm</i> of <i>fork</i> , shorter 0.066 mm. × 0.008 mm.	Length of <i>each arm</i> of <i>fork</i> , longer 0.081 mm. × 0.008 mm.
9. Ventral sucker slightly protuberant	No marked protrusion of ventral sucker on lateral view (<i>vide</i> Plate III, fig. 3 a)	Marked protrusion of ventral sucker on lateral view (<i>vide</i> Plate III, fig. 4 a)

Our measurements for the cercaria of *S. mansoni* are larger than those given by Faust, Iturbe and Gonzalez, while our measurements of *S. haematobium* cercaria are slightly smaller than the latest given by Faust (June, 1919). Regarding the details, see Plate III, figs. 3 and 4.

The anterior sucker appears to be provided with a number of papillae (about 16), which can be protruded or retracted by muscular action, and which

probably assist the cercaria to cling to any stationary object, and in this way to gain entrance through the skin of its host. Lutz (1917) has noted on the head of the cercariae a ring of six or more small points, like the crown of a trephine, which may be considered to be a boring organ. This feature is not noted by Leiper, but the Japanese authors observed it in *S. japonicum* without attaching any importance to it. The oral sucker has a posterior prolongation (pharyngeal pouch), into which the ducts of the poison or salivary-mucin glands open.

The ventral sucker or acetabulum is circular in outline and lies in the posterior fifth of the body, and when the cercaria is at rest the opening of this sucker is tri-radiate in shape. Lying immediately in front of the ventral sucker there is a small round brown pigmented simple gland of a granular consistency from which a small duct runs forward into the mouth. This can only be made out in fresh, but not in stained, specimens.

On each side of this lie the "poison cells" or salivary glands, each of which contains a well-marked nucleus, and from these individually a duct can be distinguished passing forward into the mouth. The number of these salivary-mucin glands varies in the two species, as pointed out by Faust, and which we have been able to confirm from our specimens. In *S. mansoni* cercaria (Pl. III, fig. 3) there are six pairs of glands altogether, of which two are large and clear with large nuclei and four are small and granular with correspondingly smaller nuclei. In *S. haematobium* cercaria (Pl. III, fig. 4) there are three pairs of large clear cells with acidophil protoplasm and clear cut nuclei. In front of the salivary glands the primitive nerve ganglion divides into two branches; it would appear that this structure has been mistaken for the oesophagus.

Immediately behind the ventral sucker there is a collection of densely packed nuclei, representing, probably, a primitive genital centre, and laterally placed to these are two oval excretory flame cells. The junction of the body and the tail is delimited by a membrane of oval shape and delicate consistency. The diameter of the tail diminishes towards the fork. Each arm of this fork tapers towards its termination and ends in a definite spike. The integument of the body as well as of the tail is covered with minute spines which can only be made out in the more mature cercariae, as has been described by Leiper for *S. japonicum* cercaria.

THE EXCRETORY SYSTEM.

The excretory, or protonephridial system of these schistosome cercariae appears to be identical and has been described by Cort. It consists of six pairs of flame cells arranged along the margins of the body; from these cells capillary canals arise which join collecting tubules of a greater calibre running both in an anterior and a posterior direction.

The excretory bladder consists of symmetrical convoluted tubes which project in a V-shaped manner just in front of the acetabulum (or posterior sucker), thence they run backwards and fuse at the junction of the tail to form

a single tube which extends to the posterior extremity; bifurcates once more, and opens on the tip of each bifurcation.

The *deportment* of the cercaria appears to be as follows: it generally swims with the expanded tail uppermost, and may be seen resting on the surface with the forks spread almost at right angles to the rest of the tail. When many cercariae are present in a sample of water they arrange themselves at regular intervals from one another throughout the different strata. Should the surface of the water then be disturbed, the cercariae immediately become active, and by vibratile motions of the tail make their way to the point from which the disturbance commenced.

Oxygen is necessary for their existence, and for this reason cercariae put up in hanging drop preparations in an air-tight vaseline compartment die with much more rapidity than those kept for a similar time in open glass jars.

The cercariae of *S. japonicum*, according to Leiper and Faust, are of a smaller size than those of *S. haematobium* and *mansoni*, and as in the latter species the whole body is covered with minute spines. The oral sucker is enormously developed, occupying almost the anterior third of the body. At the lip of the sucker there is a series of small tubercles and, according to Faust, the salivary-mucin glands consist of five pairs with acidophilic protoplasm arrayed around the ventral sucker.

MODE OF ENTRY INTO HOST.

We already know that once ejected from the intermediate host, the cercaria has but from 24¹ to 36 hours in which to come into contact with the skin, or mucous membrane of the upper alimentary tract of man, its definitive host.

The actual method by which penetration of the skin or mucous membrane takes place is still under investigation. Two factors are, however, probably concerned:

(1) The secretion by the cercaria of some chemical substance which has the property of dissolving the inter-cellular substance between the epithelial cells.

(2) The power which the cercaria possesses of rapidly altering its shape by contraction or relaxation of its body.

Leiper demonstrated the process by immersing a mouse for half an hour in water heavily infected with cercariae, and subsequently embedded it in paraffin. Microscopically, sections showed cercariae in various stages of transit through the unbroken skin. At the end of the half hour only a few of the cercariae were left in the water, but a great number of detached tails were observed. Warmth would appear to be the main factor in attracting the cercariae to the skin.

¹ In actual practice during the summer months in Cairo we found that the majority died after 12 hours.

After the cercaria adhered to a fixed object, such as the skin of its host, by means of the oral sucker, the tail is cast off from the body in the following manner. The mouth muscles are contracted, and this act is followed by a protrusion of the papillae of the oral sucker; simultaneously, waves of contraction pass along the body and the ventral sucker also obtains a firm hold of its victim.

Rapid vibratile side-to-side movements of the tail then take place, and the fork is opened and closed in a forceps-like manner. In a short space of time the tail parts from the body at its membranous junction and floats away.

ARTIFICIAL INFESTATION OF MONKEYS.

During the present investigation, we have produced artificial schistosomiasis in 24 monkeys.

4 were infested with *Schistosomum haematobium*.

20 were infested with *Schistosomum mansoni*.

In most cases the dual routes of infestation, *i.e.* via both the skin and upper alimentary tract, were employed, but in two, infestation was produced by infected drinking water, and in six others, by the skin route only.

Marked pruritus was a prominent manifestation in these monkeys; they scratched their skin or lips on those portions which had come in contact with the infected water. Many of the soldiers infected at Tel-el-Kebir gave such a history of itching while bathing, or immediately after leaving the water. Indeed some of them actually left off swimming on this account. In three of the monkeys, definite skin rashes were observed the day after exposure, on the areas exposed to the action of the infected water. The existence of a similar rash in *S. japonicum* has been noted by Miyagawa.

Similar phenomena were observed at the village of el Marg on the arms and legs of the Arab boys who collected snails from that locality, after immersion in the water of the smaller canals during collection of specimens. In the most heavily infected zones the itchiness was so marked that sometimes the boys voluntarily left the water on that account. In these cases, even within as short a period as 20 minutes, whitish papular elevations, the size of a small pin's head, appeared closely together over the skin of their extremities. This rash never persisted for more than 48 hours. Intense itchiness was present, and scratching sometimes led to reddening of the part due to extravasation of blood into the papules. Infested snails could always be demonstrated in large numbers in water that produced these symptoms. As a matter of fact, we were able to learn the position of several highly infected localities, by finding, from interrogating the Arab collectors, those places where they developed this pruritus during collecting.

In infestations through the upper alimentary tract the cercariae attach themselves to the mucosa of the buccal cavity either of the pharynx or of the oesophagus.

A solution of hydrochloric acid, 1 in 1000, was found to quickly kill the

most active cercariae, so it may safely be assumed that they die on reaching the stomach.

After penetrating the skin or mucosa, the cercariae are conveyed by the venous system to the right heart and so to the lungs. Here they may conceivably be temporarily held up in the pulmonary capillary bed, but eventually reach the portal vein and liver.

The lung of a monkey exposed to heavy infection three days previously showed no cercariae on section.

Once having reached the portal venules, the cercariae develop into adult male and female worms; the latter being then enveloped in the gynaecophoric canal of the former. Subsequently the paired worms travel against the portal blood stream to their ultimate destinations.

The different distribution of the paired worms in the two species of infestations is of considerable interest. In *S. mansoni* infestations, the habitat of the worm in infested monkeys was the inferior and superior mesenteric veins, and the portal veins of the liver.

In *S. haematobium* infestations, paired worms were found in portal veins of the liver, as well as in the inferior and superior mesenteric veins, *i.e.* the vesical and uterine branches. From here many wandered into the inferior vena cava, and were filtered out into the lung. This tendency was never noted in *S. mansoni*, though their lateral spined eggs have been found in those situations.

MORPHOLOGY OF ADULT PARASITES.

General characteristics common to both species of worms.

The male schistosomes are 1–1.5 cm. in length. The greatest breadth posterior to the ventral sucker is 1 mm. The anterior truncated portion is short, narrow and cylindrical, and bears suckers; the posterior part is flat and leaf-like; more posteriorly the lateral margins of this fold inwards and overlap, forming the gynaecophoric canal in which the female lies. The ventral sucker is situated just anterior to the junction of the fold. Posterior to the ventral sucker, the body of the male is covered with wart-like projections. The suckers are two in number, and are covered with papillae. The oral sucker is situated anteriorly and is funnel-shaped, with the dorsal lip larger than the ventral and communicating with the mouth.

The ventral sucker is longer than the oral, is somewhat triangular in shape, and the apex of the triangle forms the stalk of the attachment.

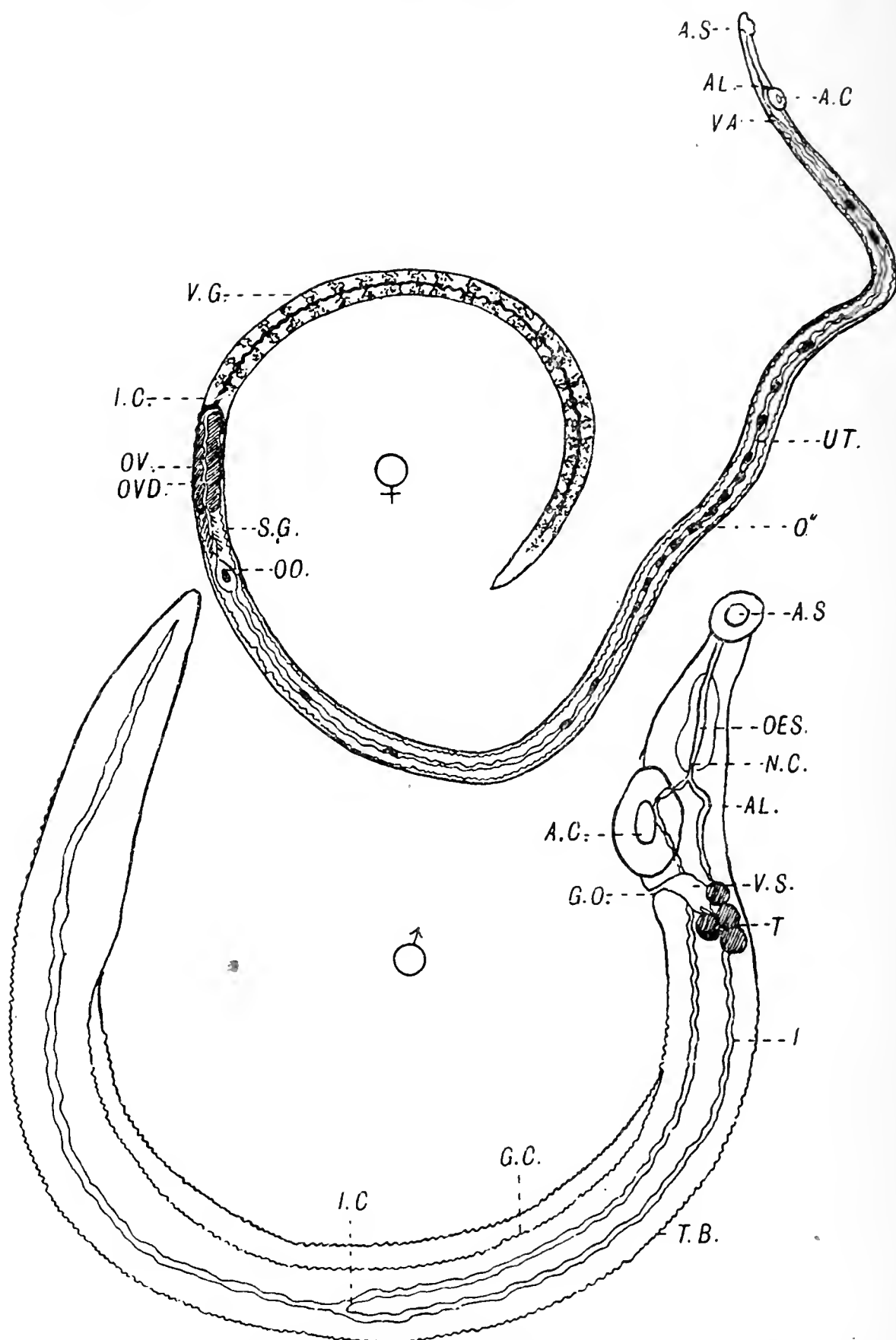
The function of this particular sucker would appear to be to fix the worm in a stable position while that of the oral sucker is essentially prehensile and alimentary.

The excretory pore is placed posteriorly and is dorsal in position. The genital opening lies just posterior to the ventral sucker in the median line.

The alimentary canal presents a mouth opening into the oral sucker and a short straight oesophagus which bifurcates just posteriorly to the ventral

sucker, and ultimately gives rise to the intestinal caeca. These caeca pass back laterally when they fuse to form a single tube which terminates posteriorly in a blind end.

The intestinal caeca are dark in colour owing to digested blood. The reproductive organs consist of irregularly rounded testes lying dorsal to



Text-fig. 1. *Schistosomum haematobium*. Adult ♂ and ♀ × 30.

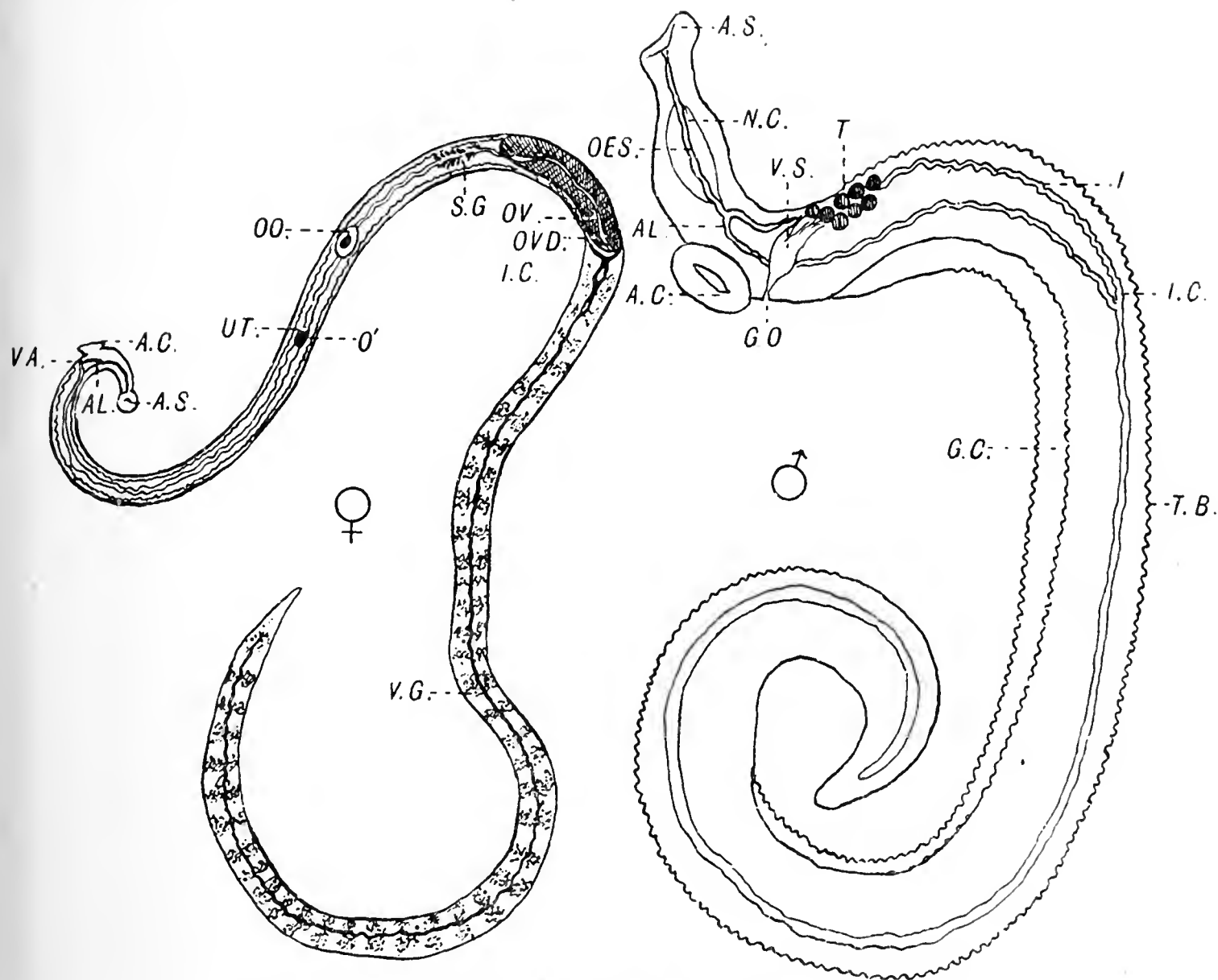
From drawings made to scale by Dr J. K. Lund.

LETTERING TO TEXT-FIGURES 1 AND 2.

A.S. anterior sucker; *A.C.* ventral sucker; *AL.* bifurcation of alimentary canal; *OES.* oesophagus; *N.C.* nerve chord; *G.O.* genital opening; *V.S.* vesicula seminalis; *T.* testes; *I.* intestine; *I.C.* union of intestinal caeca; *G.C.* gynaecophoric canal; *T.B.* tuberculations; *VA.* vagina; *UT.* uterus; *O'* lateral spined ovum; *O''* terminal spined ovum; *OO.* ootype; *S.G.* shell gland; *OV.* ovary; *OVD.* oviduct; *V.G.* vitelline glands.

the ventral sucker. Vasa efferentia lead from each testis and unite to form the vesicula seminalis. This is an elongated tube which narrows anteriorly and passes into the vas deferens, and this in turn opens on to the exterior by the genital pore by means of an ejaculatory duct.

The *female schistosomes* are cylindrical and thread-like, 1.5 to 2 cm. in length, and tapering at the anterior and posterior extremities. In breadth they are a quarter that of the male—0.25 mm. The body is smooth save towards the posterior end and on the suckers where there are papillae. They possess two suckers, oral and ventral. The alimentary canal is similar to that



Text-fig. 2. *Schistosomum mansoni*. Adult ♂ and ♀ × 30.

From drawings made to scale by Dr J. K. Lund.

of the male, the caeca uniting to form a single median tube zig-zag in appearance.

The *reproductive organs* consist of (1) an ovary, an elongated oval body lying anterior to the re-union of the intestinal caeca. The oviduct arises from its posterior end and bends forward to unite, anteriorly to the ovary, with the vitelline ducts from the yolk gland. (2) The yolk glands which occupy the posterior part of the body, lateral to the single tube formed by the intestinal caeca. (3) The shell gland which also opens near the junction of the vitelline ducts and the oviduct. (4) The uterus which is a long tube opening on to the exterior at the genital pore.

The difference in the two species *Schistosomum haematobium* and *Schistosomum mansoni* is enumerated in detail in Table III, which has been constructed after a careful study of the morphology of these two schistosomes.

Table III.

Differences observed in the two species of Schistosomum. (Text-figs. 1 and 2.)

<i>S. haematobium</i> (Bilh. v. Sieb. 1852). (Fig. 1)	<i>S. mansoni</i> (Sambon, 1907). (Fig. 2)
MALE	MALE
Length 1.5 cm.	On the average slightly shorter than <i>S. haematobium</i> , about 1 cm.
Finely tuberculated.	Grossly tuberculated.
Testes large and generally four in number, posterior to ventral sucker.	Testes small and eight in number, posterior to ventral sucker. Ventral sucker more prominent.
FEMALE	FEMALE
Length 2 cm.	Length 1.5 cm. maximum.
Ovary in posterior third of worm, in front of intestinal caeca.	Ovary in anterior half of body, in front of union of intestinal caeca.
Uterus contains large numbers of terminal spined ova with spine directed backwards (maximum number 50).	Uterus contains usually one, at most a few, lateral spined ova, with spine directed backwards.
Lateral branches of intestinal canal unite in posterior third.	The lateral branches of the intestinal canal unite in the anterior half of the worm.
Yolk glands distributed in posterior fourth of the body.	The yolk glands are widely distributed in posterior half of the body.

NOTE. From available literature it appears that worms of *S. japonicum* are of smaller size, the male being from 0.9 to 1.2 cm. long, and the female 1.2 cm. long, though Katsurada (1913) has described specimens 2 cm. and over. The ventral, relative to the oral sucker, is proportionally larger. The integument of the male is smooth, and non-tuberculated. The posterior part of the body is relatively wider, the over-lapping of the gynaecephoric canal being more extensive than in the other schistosomes.

THE HABITAT OF THE ADULT WORMS IN THE VEINS, AND THE METHOD OF DEPOSITION OF OVA IN THE TISSUES.

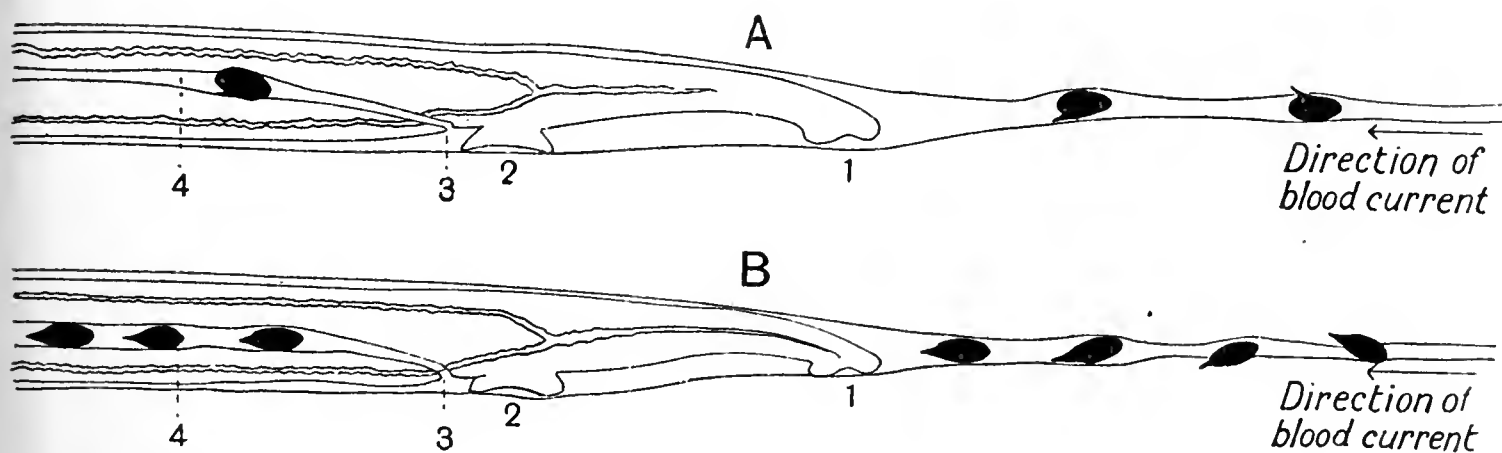
These observations were made on the adults of *S. mansoni* and *S. haematobium* lying in the mesenteric veins of anaesthetized monkeys.

The coupled worms, males and females, lying in the loops of the mesenteric veins, make their way to the finer venules, progressing by means of peristaltic movements of the body, and by action of the ventral sucker. The male assumes the chief part in this progress, the female being carried in a purely mechanical manner, in the gynaecephoric canal. Quite often, bunches of six or more coupled worms are seen progressing in one large vein in this manner.

The male adheres to the vessel wall, first by its ventral sucker, then by muscular contraction, the head lengthens out and a new grasp is obtained of the vessel wall. The anterior sucker does not appear to be used as an organ

of progression, but as an organ with an alimentary function. Where the worms do not entirely block the vessel, it is possible that their tuberculated skin enables them to maintain a stationary position against the blood stream. When desirous of depositing ova, the female protrudes herself from inside the gynaecophoric canal, and migrates in the manner described above, until she reaches a very fine branch. It is obvious that her smaller diameter enables her to do this with comparative ease, and when the diameter of the vein limits her progress she ejects her ova through her genital aperture, as shown in the diagram (Text-fig. 3, A and B). In the case of the *S. mansoni* these are few in number, but in the case of *S. haematobium* they are much more numerous. The greatest number of lateral spined ova noted in a venule has been six, whereas fully twenty terminal spined ova may occur actually jammed into one vessel. In each case the spine is directed towards the larger diameter of the vessel, *i.e.* in the direction of the flow of the portal blood.

Now the transverse diameter of the anterior truncated part of the female



Text-fig. 3. Diagram representing method of deposition of ova into the lumen of a small vein by the female and their subsequent passage through the vessel wall: **A** in the case of *S. mansoni*, and **B** in the case of *S. haematobium*.

1. Anterior sucker. 2. Posterior sucker. 3. Vaginal orifice. 4. Uterus with contained ova.

worm when stretched out in this manner becomes not very much greater than that of the deposited ovum, therefore it follows that these eggs must be in very close apposition to the walls of the vessels. By blocking the vessel in this manner the blood current in the vein, which has become stretched to accommodate the worm, becomes arrested.

When the female worm withdraws from the smaller branch vessel into which she has worked her way, towards one of larger diameter where she has left her male partner, the vessel wall immediately contracts down to its normal size and thus comes into contact with the deposited ovum; in consequence, there is every probability that the spine of the ovum will engage the vessel walls. Reflex spasm of the muscular tissue of the wall is an additional factor for ensuring this result. Apparently, after withdrawing a little, the female again becomes stationary; this act is again repeated, and in this manner a whole series of ova are deposited in small vessels giving the appearance of a string of sausages in miniature. The final withdrawal of the

female from a branch vein leads to a resumption of the blood current which impinging successively on the thin anterior ends of the ova drives them through the vessel wall into the perivenous tissues.

In the case of lateral spined ova, the marked increase in the transverse diameter of the ovum caused by presence of the lateral spine facilitates it in engaging the wall of the vessel (see Text-fig. 3).

In the case of the terminal spined ovum, unless its long axis lies mathematically parallel to that of the vessel, the impact of the blood current on its anterior end will turn it in an oblique direction. Immediately this occurs, the terminal spine will engage the vessel wall, and penetration will follow.

By these means the adult females reach the submucous tissue and the ova appear in the urine or faeces, as the case may be, within a few hours of being deposited, unless held up by fibrous tissue.

The description set forth above is not based on theory but upon the following observations:

(i) The female worm is often found separate from the male on opening the abdomen of an infested monkey under anaesthesia.

(ii) The female worms may be found singly in microscopic sections, filling up the veins of a calibre approximately equal to their own.

(iii) Direct microscopic examination of pieces of the intestine (of infested monkeys), squeezed between glass slides by means of rubber bands, will reveal the presence of ova in the vessels. Generally, several ova are deposited in a single vessel at close intervals. In many cases the diameter of this vessel is no larger than the transverse diameter of an ovum, and indeed, is often less, so that the vessel may present the appearance of a string of sausages. In some vessels ova could be observed lying in close apposition to the vessel walls, but without the spine having engaged. In others, the spines, both terminal and lateral, could be seen projecting through the vessel walls, while the bodies of the ova still occupied the lumen. In other cases ova could be observed lying free in the tissues outside the ruptured venule. The ova were always lying in the vessel with the anterior blunt end pointing in the direction of the decreasing diameter of the vessel, and with the spines in the direction of the portal blood current.

(iv) By means of a hand lens sacculations of the vessel walls could be observed corresponding with the long strings of ova referred to.

(v) Examination of the uterus of gravid females has revealed the ova *in situ*, and invariably they are placed with the spine, whether lateral or terminal, posteriorly, and the blunt narrow end of the ovum, anteriorly situated.

IV. OBSERVATIONS ON CERTAIN MOLLUSCAN INTERMEDIARIES,
ESPECIALLY RELATING TO THOSE OF EGYPTIAN BILHARZIASIS.

PLANORBIS GROUP OF SNAILS.

(1) *Planorbis boissyi*, as its name implies, is quite flat when laid on an even surface. It acts as the intermediate host for *Schistosomum mansoni*. We have found two varieties of this snail at el Marg (near Cairo). One has a dark gray or black thickened shell, often covered with whitish incrustations, the other has a light brown thin shell free from any deposits. It is the latter variety that is most commonly infested.

This is the most common snail in the sweet-water canals at el Marg, and is to be found in many places in the Delta, at Ismailia, Tel-el-Kebir, Abou Soueir, and Tanta. Agglutinated masses may be found floating down stream in the canals, and in this way, snails originally infested in the more closely populated districts may be transported over wide stretches of country.

(2) *Planorbis mareoticus* was also met with at el Marg. Its chief characteristic was its small size.

BULLINUS GROUP OF SNAILS.

The differentiation of the four species of *Bullinus* and of their many transitional forms is no easy matter, except for the experienced conchologist. The four species are *Bullinus contortus*, *Bullinus dybowskyi*, *Bullinus innesi*, and *Bullinus forskali*. In common, they all possess a non-operculated sinistral shell, that is to say, the helix runs from left to right when the apex points upwards; and the mouth, facing the observer, is situated on the left. Leiper has shown that three of these species, *B. contortus*, *B. dybowskyi*, *B. innesi*, act as intermediary hosts for *S. haematobium*. The bodies of all these snails contain a red diffusible pigment.

The only other snail commonly seen in the Canal Zone with the above characteristics is *Physa subopaca*. It is easily distinguished by its rapid movement, by the different shape of the shell which is more pointed and elongated, by the different shape of the foot, and by the absence of any red pigment. Numerous attempts to infest the *Physa* artificially failed, and over 100 specimens collected under natural conditions, and dissected, were found to be free from cercariae. No attraction for this species of snail was exhibited by miracidia hatched out of terminal spined ova, though frequent experiments were made to investigate this point.

In colour, the *Bullinus* vary from greenish black to brown. If infested with *Schistosomum haematobium*, the apical convolutions are yellow when viewed by transmitted light. If infested with *Gastrodiscus aegyptius* (Cobbold), the apical whorl is an intense black, but if infested with a yellow coloured redia (probably *Cercaria lutea* (Gilchrist)), it is bright orange. Infestations with the two latter parasites are extremely common in these snails.

OBSERVATIONS ON INFESTED SNAILS OF *BULLINUS* AND *PLANORBIS*
GENERA.

Under laboratory conditions we have frequently found that infested snails die more rapidly than normal ones, even when great care is taken regarding feeding and supply of fresh water, etc. If out of a batch of such snails 100 are dissected at weekly intervals, the percentage of those infested is found to steadily decrease. Even under laboratory conditions however, and notwithstanding a heavy infestation, certain snails have been observed to produce cercariae for many weeks. It seems probable, therefore, under natural conditions that infested snails may survive for long periods.

We have observed very definite macroscopic and microscopic changes in infested snails.

The appearance of the infested snail. After a little experience the infested snail can be recognised by the naked eye, by certain stigmata; this more generally applies to *Planorbis*. The shell itself is glossy, smooth, and shiny in appearance, and is almost invariably friable—this point alone is a good indication of probable infestation.

The infested snails are, in the majority of cases, fully adult and the hermaphrodite gland contains ripe ova, though occasionally half-grown specimens have been found infested. In those heavily infested, the golden-yellow colour, so characteristic of the liver in this condition, may be seen appearing through the shell wall itself. The organ itself is swollen and friable; it may be either ochre, bright yellow, or greenish brown in colour, and abundantly speckled with gray spots. Generally the right lobe of the liver contains the greater number of cercariae, as does also the hermaphrodite gland, which lies midway between the two hepatic lobes. The fatty degeneration of the liver, as it would appear to be, is not brought about exclusively by the bilharzia cercariae, but occurs also in snails infested with the cercariae figured in Plate IV, fig. 5.

On microscopic examination deposits of sporocysts and cercariae in the interacinous tissue are to be noted. These displace the acini of the glands and cause pressure atrophy of the parenchyma; in heavily infected livers, fully 50 per cent. of the total mass consists of sporocysts and cercariae (Pl. V, fig. 1).

Confirmatory proof that cercariae obtained from Planorbis develop only into adult worms having the morphology of S. mansoni, while those obtained from Bullinus develop only into adult worms having the morphology of S. haematobium.

Monkeys were infested in the following way. A snail's liver, which was found microscopically to contain a large number of mature cercariae, was placed on a shaved area of the leg or abdomen of the monkey, and was permitted to remain in contact with the skin for from five to twenty minutes.

After an average period of six weeks, these monkeys became very thin and

debilitated, and were then chloroformed. The mature worms were then extracted in large numbers from the liver and mesenteric veins.

Both sexes of the worms were then found to be adult, the uterus of the females containing ova. Specimens were measured while alive, preserved by fixing in hot 70 per cent. alcohol, and then cleared in creosote. The morphological differences verified in this manner entirely corroborate those detailed by Leiper (1916) and on these observations Table III has been compiled.

Transmission experiments under artificial conditions with the object of determining whether Planorbis acts as a strictly selective intermediate host for S. mansoni and Bullinus (Species contortus and dybowski) for S. haematobium.

For this purpose experiments were undertaken in order to breed *Planorbis* in the laboratory, and thereby to ensure its freedom from previous infestation. This was found to be easily feasible, and a number of laboratory-bred snails were obtained from adults, which had been placed in a vessel three months previously. It was found that on an average, *Planorbis* took, in the atmosphere of Cairo in the spring and summer months, about three months to attain to maturity.

Thirty-six of these snails were placed in a vessel together with the centrifugalized urinary sediment containing ova of *S. haematobium*. On examination, two months later, a batch of nine were dissected and no cercariae were found. The remainder were then placed together with lateral spined ova of *S. mansoni*, occurring in the urine of an infested soldier. Subsequently after a further period of six weeks 21 per cent. of the survivors were found to be heavily infested with cercariae which gave measurements identical with those already recorded for *S. mansoni*.

Similar attempts to breed *Bullinus* in captivity were not attended with success, but a large supply of these snails was obtained in August, 1917, from a sedimentation tank, fed by filtered water from the sweet-water canal where there was no chance of faecal or urinary infection; in order to ensure that these snails were not infested by cercariae of any description, one hundred were dissected and found to be normal. Attempts were then made to infest a large number by means of lateral spined ova obtained from the urine with entirely negative results.

THE SEASONAL INCIDENCE OF INFECTIVITY OF *PLANORBIS* AND *BULLINUS* WITH THE TWO SPECIES OF *SCHISTOSOMIDAE*.

The accompanying tables show that considerable numbers of snails all obtained from the same locality (el Marg) were dissected for twelve consecutive months, and one remarkable fact emerges, namely, that the largest number harbouring cercariae of both forms occurred in the late autumn months, and especially in December. It was further noted that in these months

(late autumn) the livers of the infested snails harboured full grown cercariae, while in the early spring, immature cercariae and sporocysts were the rule rather than the exception.

Table IV.

Seasonal incidence in infestation.

Month	<i>BULLINUS</i>		<i>PLANORBIS</i>	
	Number of snails dissected	Percentage infested	Number of snails dissected	Percentage infested
1917				
May	51	1.9	280	37
June	27	0.0	572	19
July	140	0.7	288	29
August	57	1.7	567	8
September	93	0.0	689	10
October	No record	No record	920	6
November	50	4.0	1468	30
December	362	9.0	379	54
1918				
January	140	0.0	150	24
February	72	3.0	100	10
March	69	0.0	252	32
April	41	2.5	124	28
Total*	1102	1.9	5789	18

* These percentages have been worked out from the total number of specimens found infested each month.

During the period of maximum infestation the canals are full of irrigation water resulting from the annual rise of the Nile—this would appear to be one factor.

During the months of January and February, according to our observations, a large number of the adult snails die off, and, in fact, the survivors are very difficult to find as they remain inactive at the bottom of the pools. In April and May these canals are periodically flushed with a large addition of stored water, and it is at this time that the maximum breeding of *Planorbis* takes place. For then large floating masses of young *Planorbis* adhering to the weeds collect in stagnant backwaters in the native villages, and, owing to the proximity of the native houses, they stand a very good chance of becoming invaded by miracidia.

The breeding season of *Bullinus*, on the other hand, from observations we were able to make in the sedimentation tanks at Kantara, would appear to be the months of July and August, when the water in most of the smaller canals is at low ebb. At this period also, this snail is most active and appears on the surface of the water, where it is scarcely ever found at other seasons. Owing to the small volume of water in the canals it is legitimate to assume that this is the season wherein the maximum opportunity of being brought into close contact with the miracidia of *S. haematobium* occurs.

The same rule that appertains to bilharzia also appears to hold good for the developmental stages of other trematodes. The statistics in Table V. show that the *Bullinus* harbour the greatest number of these also in the month of December. The increase during this month is due to the increase of pigmented cercariae of *Gastrodiscus aegyptius* which is a parasite of the buffalo. According to our experience, this parasite is found mostly at this period of the year, and not during the height of summer, see Table V.

Table V.

Dissections of Bullinus contortus and Bullinus dybowski for presence of other cercariae (Cercariae of Gastrodiscus aegyptius, and those figured in Plate III, figs. 5, 6, 7).

Month	Number of snails dissected	Percentage infested as above
1917		
May	51	39
June	27	18
July	140	31
August	57	29
September	93	20
October	No record	No record
November	No record	No record
December	362	75
1918		
January	140	57
February	72	70.8
March	18	70
Total	960	54

In summarizing these results we might state:

(1) That in contradistinction to the observations of Looss we have found throughout the winter months, marked growth and multiplication of laval trematodes—*S. haematobium*, *S. mansoni*, *Gastrodiscus aegyptius*, and others.

(2) That while the month of maximum infectivity for both species of Bilharzial cercariae was December, there was definite evidence to show that *endemically areas are potentially infective throughout the whole year.*

DESCRIPTION OF NON-EYED, FORK-TAILED CERCARIAE FOUND IN FRESH-WATER SNAILS IN EGYPT.

Only two of these were found and both are obviously not connected in any way with disease in man; one a very small fork-tail cercaria in *Planorbis mareoticus* from the Zoological Gardens, Giza. The other was obtained in 4 per cent. of a batch of *Bullinus dybowski* in the sweet-water canal by the Little Bitter Lake. We have also been able to examine the cercaria of *S. spindale* from India, kindly sent by Lt-Col. Glen Liston, I.M.S.

1. Bilharzia-like cercaria from *Planorbis mareoticus* (Plate III, fig. 5).
The measurements of this cercaria were as follows:

Total length	0.296 mm.
Body	0.119 mm. \times 0.043 mm.
Tail	0.177 mm.
Fork	0.044 mm.

This form differs materially in having a marked and elongated oral sucker, armed with numerous and prominent papillae. Pigmented eye spots were seen in the specimens collected by Leiper, but were not present in those we examined, as perhaps they were too immature. The ventral sucker is round and comparatively large. In front of it lie two pairs of salivary-mucin cells; the tail is rather broad and provided with short forks. The integument is covered with minute spines. The sporocyst is an oblong oval measuring 0.119 mm. by 0.102 mm. filled with granular germ cells and is easily dissected out from the liver substance (Plate III, fig. 6). According to Leiper this cercaria is probably the larval form of the *Bilharziella* of the duck.

2. Bilharzia-like cercaria from *Bullinus dybowskii* (Plate III, fig. 7).
The measurements of these cercariae were as follows:

Total length	0.311 mm.
Body	0.119 mm. \times 0.031 mm.
Tail	0.192 mm.
Fork with cuticular expansion	0.150 mm.

This can easily be distinguished from the true Bilharzia cercaria by its narrow elongated head, and very markedly forked tail with its cuticular expansions. Each arm of the fork approximates that of the total length of the tail. The oral sucker protrudes slightly and is armed with many minute papillae; there are three pairs of salivary-mucin cells around the ventral sucker, and there is a marked posterior expansion of the oral sucker and a rudimentary pharynx. The body and tail are covered with minute spines.

The sporocysts are elongated finger-like structures (see Plate III, fig. 8), apparently emanating from a common centre. In the outer layer of the cyst are numerous amber coloured granules. Individual cysts vary in size, the largest measure 0.678 mm. by 0.075 mm.

This cercaria appears to us to be identical with *Cercaria gladii* from *Isodora schakoi* of S. Africa described by Cawston and recently figured by Faust (1919). Our measurements are, however, smaller than his.

Both these cercariae differ materially from the fork-tailed cercariae with well-marked pharynx, but without eye spots, sent to us by Lt-Col. Glen Liston, I.M.S., from *Planorbis* in India and which has been found to be the larval stage of *Schistosomum spindale* of the goat (Liston, 1918). These measure about 0.420 mm., total length, and roughly resemble similar organisms figured

by Cawston (1915, 1917) as occurring in *Physopsis africana*, though his figures lack accuracy and detail. The measurements of this Indian cercaria we procured are as follows:

Total length	0.420 mm.
Body	0.162 mm. \times 0.047 mm.
Tail	0.260 mm.
Fork	0.092 mm. \times 0.021 mm.

For the purpose of comparison, and to aid future workers, a short description is given of other cercariae found in *Bullinus*, *Planorbis* and a few other common species of fresh-water snails in Egypt, as figured in Plate III.

Plate III, fig. 9. *Cercaria cellulosa*, a microcotyledonous distome cercaria (spec. inq. Looss, *Rech. sur la faune parasit. de l'Egypte*, p. 227, Pl. XIV, figs. 159, 161). Small, hyaline, very active and found in 10 % of *Cleopatra bulimoides* from Tel-el-Kebir. Looss found them also in *Melania tuberculata*.

The anterior sucker is provided with a hyaline dart. Opening out into it are two pairs of well-marked cephalic glands. The ventral sucker is round, and is situated immediately posteriorly to the central line of the body; laterally placed to it, are collections of genital cells. The excretory vesicle is apparent in the posterior part of the body. The sporocysts are round, or sacciform (Fig. 10) and are unpigmented.

Plate III, fig. 11. Microcercous distome cercaria found commonly in liver of *Limnaea* (mostly *L. caillaudi* and *L. truncatula*) resembles *Cercaria pusilla* spec. inq. of Looss, shows two pairs of cephalic glands with ducts opening into oral sucker, a muscular pharynx and well-marked ventral sucker. Excretory bladder situated at the posterior part of the body, and two excretory canals opening into corresponding vesicles. In the oral sucker is situated a hyaline dart.

Plate IV, fig. 1. Under this heading are included probably amphistome cercaria of three species as described by Looss. They are of a large size, about 0.84 mm. in length, by 0.427 mm. in breadth. The body is oval and almost circular, and in the more mature forms is so darkly pigmented that no details of structure can be made out. They resemble indeed, both in size and movements, minute tadpoles. They are found most commonly in *Bullinus*—about 5 %, but were occasionally seen in *Planorbis boissyi* also, as well as in *Limnaea caillaudi* and *Cleopatra bulimoides*. The pigmentation of the body seems to commence from two eye spots situated in the anterior third of the body and thence to radiate along the excretory and alimentary canals till the whole body is thus pigmented. The anterior sucker is round and smaller than the posterior, which is also circular and muscular, and is situated at the extreme posterior end of the body. The oral sucker leads into a muscular oesophagus, and eventually into a semicircular branched alimentary canal. The excretory system is represented by a series of refractile globules passing in a circular manner from the anterior to the posterior sucker. The tail is elongated and tapering. These cercariae are probably larval stages of *Gastrodiscus aegyptius* (Cobbold) of the horse, of *Gastrothylax gregarius* (Looss) and of *Amphistomum conicum* (Rud.) from the stomach of the buffalo.

Plate IV, fig. 2. Mature redia of Fig. 1; surprisingly small considering the size of the cercariae. They are obtuse oval in shape and hyaline without any protrusion or appendix and measure 0.77 mm. by 0.13 mm. and show large germinal masses in their cavity. At the posterior pointed extremity there are some well-marked germiniferous cells.

Plate IV, fig. 3. This is a monostome cercaria, the *Cercaria pleurolophocerca* of Sonsino (1895) and Looss (1896). We found it occurring in *Melania tuberculata*, both from el Marg and Tel-el-Kebir. Its total length is 1.00 mm. The body is 0.27 mm. by 0.08 mm. The tail is 0.49 mm. in length and is provided with a lateral cuticular expansion. In shape the body is an elongated oval tapering towards the anterior sucker. The cuticle is clothed externally by a number of minute

spicules. The circular oral sucker opens into a short muscular pharynx. No more of the alimentary canal can be made out, and according to Looss, this is absent. The remainder of the body is occupied by eight pairs of large cephalic glands whose numerous ducts open into the mouth cavity. Situated in the posterior sixth is an oval structure with cellular outline and an absence of a muscular rim; this was thought by Sonsino to be the ventral sucker, but Looss pointed out (*op. cit.* p. 209) that it represents the excretory vesicle and that the cercaria is probably the larval form of some monostome. In front of this excretory vesicle is situated a round well-marked collection of genital cells. The tail is long and tapering and in the more mature forms is provided with a lateral cuticular expansion as is shown in the Plate.

The rediae are sausage-shaped striking objects, 1.055 mm. in length and 0.150 mm. broad, and possess a muscular anterior sucker. They are granular in appearance and contain a number of cercariae in all stages.

Plate IV, fig. 4. Distome cercaria from liver of *Cleopatra bulimoides* commonly found at el Marg and at Tel-el-Kebir. This is evidently the *Cercaria distomatosa* of Sonsino (1895). Rediae of this species are large and pigmented and form very prominent objects. The whole body appears granular, so occupied is it with cystogenous cells that it is only with difficulty that the finer points of internal structure can be made out. The ventral sucker (acetabulum) is muscular and prominent; the oral sucker is pitted round its margin with numerous small orifices, said by Looss to be the openings of many small ducts. The pharynx is continued into a muscular oesophageal bulb. Posterior to this, the two blind sacs of the intestinal canal branch off and run into the posterior end of the body. The excretory system is represented by two fine excretory ducts, which run from the ventral sucker and open into a vesicle at the posterior end of the body. The tail is blunt-ended and does not move independently when the animal is alive. The extremity of the tail is rounded and is split for a short distance at its termination. The excretory canals open bilaterally on the anterior portion of the tail.

Plate IV, fig. 5. Very active amphistome cercaria commonly found in *Bullinus*, *Planorbis* and once in *Physa*; apparently not described by Looss. In the former snail from el Marg as well as from Tel-el-Kebir 31% were found to be naturally infested. The body is full of cystogenous cells. The oral sucker is round with an oval termination. There is a large muscular pharynx which imparts a lobulated appearance to the anterior end of the body. This is bordered on one side by a string of glands, the canals of which run forwards and open laterally into the mouth. There is a straight oesophagus which branches into two ill-defined alimentary caeca anterior to the ventral sucker. The branches of the alimentary canal run far forward, and also for a short distance towards the posterior end of the body. The ventral sucker is large, prominent and muscular; on its anterior margin there opens a small duct derived from a collection of cells (probably genital) situated at its posterior border. The tail which is equal in length to the body, is long and tapering, and contains the efferent excretory canal. This cercaria is often found encysted in the liver of *Bullinus* and *Planorbis*, especially in the winter months (*Plate IV, fig. 6*).

The redia and cercariae appear to resemble those described by Gilchrist who has worked out their life history in S. Africa and finds them to be the larval stages of *Distomum luteum* in the intestine of the frog, but our measurements are considerably larger than his.

This is possibly also the *Cercaria pigmentata* described by Sonsino.

Plate IV, fig. 7. The redia is a very striking object. The infested livers in which they are found are of a bright orange colour, and the organ appears to be packed with them. Directly the shell of such an infested snail is incised, these prominent objects can be seen floating out of the breach. The body wall of the redia is studded with bright orange pigment granules. The length is about 1.43 mm. There is a large anterior muscular sucker, and near the posterior end there is generally a lateral protrusion or appendix. The interior of the redial sac is filled with young and active cercariae. (Corresponds with that of *Distomum luteum* of Gilchrist.)

For compiling this section the works of Cort on N. American Cercariae and of Faust on those of S. Africa have been consulted.

V. CONCERNING INVESTIGATIONS OF THE SWEET-WATER CANAL TO WHICH INFECTION AMONGST AUSTRALIAN AND IMPERIAL TROOPS WAS TRACED, AND THE CONCLUSIONS BASED UPON THEM.

The following incidents form a very remarkable and practical corroboration of Leiper's work.

In October and November, 1916, several cases of bilharziasis were admitted to No. 14 Australian General Hospital. In these, examination not only revealed lateral spined ova in the faeces, but also terminal spined ova in the urine. It was therefore suggested as possible that most of the cases from Tel-el-Kebir in Lawton's series, to which reference will be made later, were mixed infestations. As no *Bullinus* had been found, when inspection following this outbreak was made, we re-investigated the Rifle Range Canal at Tel-el-Kebir, and succeeded in demonstrating *Bullinus* infested with *Schistosomum haematobium*, as well as *Planorbis* infested with *Schistosomum mansoni*.

In addition it was found that the fellaheen in the adjacent fields were passing many lateral spined ova in their faeces, terminal spined ova were found in the urine of two out of three cases examined. With the cercariae from infested *Planorbis* gathered at Tel-el-Kebir, we succeeded in infesting monkeys, and thus proved conclusively the infectivity of the canals in this area.

A careful analysis of the histories of patients admitted to Hospital suffering from bilharziasis, soon led to the conviction that other areas than Tel-el-Kebir were responsible for infestations amongst Australian Light Horse. Pools at Serapeum and Abou Soueir were suggested as sources of infestation. Further, the cases from Serapeum were all simple infestations with *Schistosomum haematobium*, while those from Abou Soueir were infested with both species. It was possible to predict therefore, on the basis of Leiper's works, the presence in any particular area of *Bullinus* or *Planorbis*, or of both. In every case subsequent investigations proved the truth of this prediction.

Table VI.

Canal zone	Infective bathing areas	Troops affected	Total Number of cases	Type of infestation			Species of snail found in pool and locality	
				<i>Schist. mansoni</i>	<i>Schist. haematobium</i>	Double infestations	<i>Planorbis</i>	<i>Bullinus</i>
Zag-a-zig to Ismailia	Tel-el-Kebir	Australian Light Horse	49	6	10	33	present	present
Zag-a-zig to Ismailia	Abou Soueir	"	6	...	4	2	present	present
Kantara to Suez	Serapeum	"	14	14	absent	present
Upper Egypt	Deirut	"	11	...	11	...	absent	present
Upper Egypt	Fayoum	Imperial Mounted Yeomanry	6	...	6	...	absent	present

NOTE. The actual pool at Deirut in Upper Egypt was not investigated, but reference to the literature shows that snails of the *Bullinus* species are found there, but not those of *Planorbis*. McCallan (1915) was able to show a 25 % infection with *S. haematobium* in the country district around Deirut.

An analysis of Table VI (attached) affords a remarkable proof of Leiper's work in demonstrating the specific relation of the two types of *Schistosomidae* and their intermediary hosts.

A description of the following bathing sites well illustrates the variable modes in which infection may occur.

1. At Tel-el-Kebir the troops bathed in a pool formed by the expansion of a narrow sweet-water canal (Pl. V, fig. 2).

2. At Abou Soueir they became infested by swimming in a much wider and deeper sweet-water canal.

3. At Serapeum they became infested by washing in a shower bath under an overflow pipe which conveyed water from the adjacent sweet-water canal.

(1) *Pool at Tel-el-Kebir* (Pl. V, fig. 2). Close to the Rifle Range at Tel-el-Kebir and running on the edge of the cultivated land between it and the desert, is a fresh-water irrigation canal not more than eight feet across at its widest, nor more than three feet deep at any part. There is a clump of shady fir trees near the Rifle Range Road, and at this point is a small sluice gate, beyond which the canal widens out into the pool in question. It is about 60 feet long and 30 feet wide by four feet deep. Beyond this the canal runs on again, lined at this point with reeds and rushes. After a morning's musketry, it was a common practice for the troops to have lunch under these trees, and many bathed in the pool, while others drank the canal water. In this way the disease was contracted.

On investigating this bathing pool and the canal, we found many snails of the species *Planorbis boissyi* floating down stream, attached to débris or joined together in masses to form rafts. It was the predominant snail of the molluscan fauna of this area. Examination of the submerged rushes and the stems of the water-grasses brought to light *Bullinus* snails in quite large numbers. A collection of snails of both kinds was made and on dissection, the cercariae, in one case of *S. haematobium*, and in the other of *S. mansoni*, were easily demonstrated. Thus, out of 513 snails of the species *Planorbis boissyi* five, or 1 per cent. were found to be infested, while of 190 specimens of *Bullinus* two, or 1 per cent. (approximately) were found to be in a similar condition.

The water level of this pool was found to vary on different days, as did also the molluscan fauna it contained. From this it was evident that its potentiality as a source of infection could vary within wide limits in quite a short space of time. In some such manner we may account for the fact that while the great majority of cases became infested with both species, a few acquired only *S. mansoni* and fewer still *S. haematobium* alone.

A point of great interest was the rapidity and comparative ease with which many of these molluscs, particularly *Planorbis boissyi*, travelled over quite wide areas by adhering to floating débris. It seems probable from this that snails originally infested from human excreta in the densely populated regions like Zag-a-zig, and perhaps even the environs of Cairo itself, through which the

canal passes, may be widely distributed over the less densely populated agricultural areas of the Delta. The flooding of practically the whole of the Delta, which takes place annually, is no doubt a very potent factor in the spread of the disease.

(2) *The Canals at Abou Soueir.* During the summer of 1916, a troop of Light Horse engaged in patrolling this sector was camped on the banks of the canal. In the hot evenings the men were in the habit of swimming in this canal, which is the second largest in the vicinity. It is deep enough for swimming and is from 40 to 60 feet in width, and its banks are lined with reeds and rushes. Investigation showed the presence of snails of the following species: *Bullinus contortus*, *Bullinus dybowski* and *Planorbis boissyi*.

As in the Tel-el-Kebir group, so in this series also, two of the cases were found clinically to be double infestations, while four were infested with *S. haematobium* only.

(3) *Serapeum (Deversoir).* Troopers of the Australian Light Horse stationed here were in the habit of bathing in the Suez Canal, which at this point enters the northern end of the Little Bitter Lake. After their swim, it was customary for a number of them to cross a belt of marshy land, about 50 yards wide, for a final shower in the fresh water of an over-flow pipe from the sweet-water canal. For this purpose they stood in a small pool about 3 feet in diameter, by 2 feet deep, formed by the falling water. In this way most of them contracted bilharziasis.

Subsequent investigation of the canal from which the pipe was leading, of the pool itself, and of the adjacent canals, revealed the presence of *Bullinus*, and of *Physa*, but only three specimens of *Planorbis boissyi* were found after an exhaustive search. Amongst the cases of bilharziasis from this zone, no infestation with *S. mansoni* was detected, though the faeces were most carefully examined in every case.

(4) *The Fayoum.* In 1917 a very considerable number of urinary cases (*S. haematobium*) occurred amongst the Yeomanry detachments; the infestation being originally contracted in 1916. Eleven of these cases have come directly under our personal observation. Terminal spined ova were found in their urine, but lateral spined ova were never demonstrated in their faeces.

In this district we found that *Bullinus contortus* was widely distributed, but *Planorbis boissyi* was never observed, after exhaustive search on two separate visits to the ponds, ditches and canals of this oasis.

The Village at el Marg. Before concluding this section the following observations made at el Marg (Delta district) situated 12 miles north-east of Cairo will be included.

Although we have no statistics of white troops billeted in this locality, the relationship between the molluscan fauna, and the types of bilharziasis occurring in that area, has been noted.

In the birkets and canals large numbers of *Planorbis boissyi* are always to be found, and greatly out-number any other species of fresh-water snail. *Bullinus*, on the other hand, is not so common. On reference to Table IV it will be seen that from over 5000 dissections of these snails, 18 per cent. were infested with *S. mansoni*. On the other hand, in over 1000 dissections of *Bullinus*, only 1.9 per cent. were found to be infested with *S. haematobium*.

In el Marg at any rate it would seem that the occurrence of rectal bilharziasis in man ought to be much more common than that of urinary form. As a matter of fact both rectal and vesical forms are exceedingly frequent amongst the native population, though from its situation the former bilharziasis (*S. mansoni*) requires more painstaking observation for its detection.

A glance at the map of Egypt will show that the bathing sites where troops acquired infection are widely spread over various stretches of fresh water, including those of both Lower and Upper Egypt.

Thus, Tel-el-Kebir and Abou Soueir are on the Zag-a-zig to Ismalia canal; Serapeum is situated on the sweet-water canal between Kantara and Suez; Deirut and the Fayoum are both in Upper Egypt.

They therefore serve, in a general way, as excellent indications of the potentialities of these particular spots.

CONCLUSION.

As a result of this study of the clinical types of bilharziasis originating in a given locality, and from subsequent investigation of the molluscan fauna of that area, we are presented with a remarkable corroboration of Leiper's work. The specificity of these two species of snails for their respective parasites has, in this fashion, been repeatedly proved.

It is surely very satisfactory to be able to substantiate that the distribution of the disease in man corroborates in such a remarkable way the experimental facts observed in the laboratory.

Wherever the Planorbis is the predominant species there will rectal bilharziasis be found, and wherever Bullinus abounds there the urinary form of the disease will be the most prevalent.

The statistics given in Table VI show how heavily parts of the Delta district are infested with *S. mansoni*. From these statistical studies, from clinical observation, and from other data, we are firmly convinced that intestinal bilharziasis (*S. mansoni*) is much more prevalent amongst the Egyptian population of Lower Egypt than is at present recognized.

Intestinal bilharziasis is more difficult to diagnose clinically for the following reasons:

(1) Intestinal symptoms in man are frequently latent, and when present do not, in a tropical country, attract so much attention as do urinary ones.

(2) Many more ova are produced by *S. haematobium* than by *S. mansoni*, and therefore the former are much more easily found under the microscope in routine examination of the discharges.

Distribution of fresh-water snails in localities visited in Egypt.

El Marg near Cairo	{	<i>Planorbis boissyi</i> (common), <i>Bullinus contortus</i> , <i>dybowski</i> , <i>innesi</i> , <i>forskali</i> , <i>Physa subopaca</i> (scarce), <i>Vivipara unicolor</i> , <i>Bythinia senaarica</i> , <i>Cleopatra bulimoides</i> (very common), <i>Melania tuberculata</i> , <i>Limnaea caillaudi</i> (scarce).
Zag-a-zig to Ismailia	{	<i>Bullinus contortus</i> and <i>dybowski</i> , <i>Planorbis boissyi</i> , <i>Limnaea caillaudi</i> , <i>Cleopatra bulimoides</i> , <i>Physa subopaca</i> , <i>Melania tuberculata</i> , <i>Planorbis mareoticus</i> .
Kantara Tanks		<i>Bullinus dybowski</i> , <i>Physa subopaca</i> .
Southern Canal Zone	{	<i>Bullinus dybowski</i> (much the commonest species), <i>Bullinus contortus</i> (rarer). Very few <i>Planorbis boissyi</i> . Amongst 1000 <i>Bullinus</i> there were only three <i>Planorbis</i> .
Cairo	{	(1. Head Storage Reservoirs. Abbassia (unfiltered water supply) water collected directly from the Nile. <i>Bullinus contortus</i> (very common). <i>Bullinus dybowski</i> . <i>Limnaea caillaudi</i> . <i>Melania tuberculata</i> . <i>Planorbis boissyi</i> .
		2. Snails from unfiltered water-pipes. <i>Cleopatra bulimoides</i> . <i>Melania tuberculata</i> .
		3. Public Ponds. <i>Limnaea caillaudi</i> .
		4. Snails from receding Nile at Agu Baba in mud. <i>Vivipara unicolor</i> . <i>Cleopatra bulimoides</i> . <i>Bythinia senaarica</i> . <i>Melania tuberculata</i> .

VI. THE INCIDENCE AND ORIGIN OF BILHARZIAL INFECTION AMONGST THE CAIRENES.

As Australia is faced with the potential danger of outbreaks of bilharziasis, the following observations on the manner in which the natives of Cairo are infested may be of importance.

Looss, in an investigation of the percentage of infested children in Cairo, found 33 per cent. of the boys at a certain school to be infested with *S. haematobium*. In 1908, Mrs Elgood showed that 27.5 per cent. of the girls attending a school in Cairo were similarly infested.

The supporters of the "Looss hypothesis" maintained, that as the children had never been out of Cairo, and as there was a common filtered water supply to the white and native population of Cairo alike, direct miracidial infestation was the only explanation that would account for the distribution of bilharziasis amongst them.

In criticizing the supporters of the "Looss theory," Leiper pointed out the presence of a second water system carrying unfiltered water from the Nile, and used ordinarily for supplying public and private gardens. This water,

on account of its cheapness, was being largely used by the natives for domestic, drinking, and other purposes.

At the request of Dr Todd, the Director of the Public Health Laboratories, we inspected the storage reservoirs of this crude unfiltered water supply derived directly from the Nile.

In the two main storage tanks *Bullinus* were found on the green water-weeds growing below the surface. In a small adjacent tank, 15 feet in diameter, 250 *Bullinus* were collected in a few minutes from the inlet pipe that comes from the Nile.

In the old disused filter beds near those at present in use, many thousands of shells of *Bullinus*, and some of *Planorbis boissyi*, were found.

These findings suffice to explain the incidence of bilharziasis amongst the native children of Cairo who have not been outside the City itself, and prove the danger of this unfiltered water supply to the whole community. The molluscan fauna in the ponds and fountains of public gardens of Cairo are also derived from this water supply. We have frequently noted that either the spawn, the young, or the adult snails themselves can withstand the water-pressure in the piping. Thus at el Marg, infested snails may be obtained in quantity in the railway tanks which derive their water supply from adjacent canals.

While the infectivity of the Nile water has never been demonstrated, our observations support those of Leiper on the subject. The presence of snails in the pipe conveying water from the Nile, and the large number of shells left in the mud on the banks when the water recedes, prove the wide distribution of fresh-water snails in that river. Felucca men (boatmen) state that snails are to be found adherent to their boats if they have been anchored for any length of time during the summer months. Several years ago an outbreak of bilharziasis occurred amongst soldiers who had bathed in a specially constructed floating wooden bath, in the Nile at Kasr-el-Nil bridge. Here the conditions for the concentrated action of cercariae were ideal, for snails adhering to the side of the framework nearest the current would create a zone of infectivity in the water immediately beyond.

All along the Nile banks deposits of human faeces may be observed, similarly urinary contamination occurs, owing to the un-hygienic habits of the natives.

Along the banks where the reeds and grasses cause the water to become stagnant, snails may be observed, and it is here that the miracidia from infected excreta have every chance of finding their intermediary hosts. Native water-carriers fill goat skin bags with this water, and sell it to the native population at a very cheap rate. It is used by them for drinking and domestic purposes. This practice may constitute another very real danger to the community.

VII. SUGGESTIONS FOR PROPHYLAXIS.

Prophylactic measures necessarily resolve themselves into two lines of action.

1. Destruction of ova in the excreta prior to contamination of the water.
2. Destruction of the molluscan intermediary hosts of the Schistosomes and the purification of contaminated water supplies.

(1) *Destruction of ova in excreta prior to contamination of the water.* In any highly civilized community an efficient system of sanitation would markedly lessen, if not entirely eradicate, the disease.

In Egypt, however, the Arab inhabitants are far from being highly civilized, and their ideas of sanitation are rudimentary in the extreme, if not entirely lacking. The water in the canals is being constantly contaminated with urine and faeces, and in consequence miracidia are abundant. Especially is this the case in the environs of large cities such as Cairo, Zag-a-zig, etc. With the means at present at the disposal of the Government the problem of educating the fellaheen on sanitary lines is beyond solution; it must be many years, one is tempted to say, centuries, before prophylaxis based on a system of rational sanitation will be practicable.

Introduction of bilharziasis into countries in which it is at present not endemic.

One of the chief topics of medical interest at the present moment is to prevent the introduction of bilharziasis into countries where it is not at present endemic, but where the fresh-water snail fauna may be capable of acting as intermediaries and the climatic conditions are suitable.

Something of this sort seems to have happened in the analogous case of the liver fluke (*D. hepaticum*) when introduced into Australia together with its vertebrate host—the sheep.

The ideal method to prevent the introduction of this disease would be to confine carriers of Schistosome ova to sequestered regions where their dejecta would be incapable of reaching any water supply in which the suitable fresh-water mollusca could live.

One need hardly point out that the systematic microscopic examination of the urine and faeces of all persons returning from an infected country such as Egypt, to an uninfected one, such as Australia, is almost an insuperable task; and Australia is not alone in facing the problem to-day, for the same condition applies even more strongly to India, where apparently the agricultural and climatic conditions are favourable and the snail fauna similar to that of Egypt but where up to the present transmission experiments with the local *Planorbis*¹ have failed.

¹ Private communication from Prof. Annandale and Dr Kemp.

As we suggested in 1916, the ideal procedure would be to confine those cases known to be infested with bilharzia that have returned to Australia to sewerage areas, at all events till they have learned to appreciate their potential danger to any community. These suggestions, we understand, have been carried out.

These measures alone might appear sufficient to prevent the disease ever becoming endemic in Australia, but such is really not the case. We have met with fully 20 cases styled "Latent Bilharziasis." These patients have either no symptoms at all, or their symptoms have been so slight as to be entirely disregarded. Nevertheless, the examination of their excreta has revealed the presence of lateral or terminal spined ova. It is in infestations with *S. mansoni* that latency is most frequently seen. Most of these "latent" cases were diagnosed in a routine examination of a large number of sera by means of the specific complement deviation test for this disease. They have been more fully dealt with in previous publications. (*Journ. Roy. Army Med. Corps*, iv. and vi. 1919.)

From the above it will be seen that the existence of the disease in people who are unaware of, or neglect the signs of, its presence, considerably complicates the problem of the prevention of the spread of this disease into a community. It is the carrier problem over again!

(2) *The destruction of intermediary hosts.* The destruction of the molluscan fauna of a country, such as Egypt, would lead to the disappearance of bilharziasis in man and of a number of "trematode" diseases affecting animals.

In a country with an intensive system of irrigation, such for instance as Egypt, the molluscan fauna becomes a universal and persistent scourge. Many measures have been proposed for the eradication of the intermediate snail hosts.

Dr C. Todd, the Director of Public Health, informs us that several years ago the possibility of reducing the molluscan fauna by means of ducks and wild fowl was carefully considered, but the idea was rejected as impracticable for Egypt.

More recently, Major T. Cherry has advocated this method as applicable to Australia. While it is possible that the presence of ducks and other wild fowl might materially help to diminish the number of molluscs in localized water areas, it is far from being the solution of the problem of Egyptian bilharziasis, and at most can only be regarded as a possible accessory measure.

In 1915, Leiper, in his "suggestions for the eradication of the disease in Egypt," proposed that the annual rotations in the supply of water enforced by the Government from April to August in Lower Egypt, should be utilized to destroy the molluscan fauna.

During the periodical stoppages of water supply for 15 days, the canals become dry except for small puddles. By the systematic use of destructive chemical reagents, Leiper suggested that the number of contained molluscs could be considerably reduced. It may be that methods of this sort, if they could be attempted, would be possible for the smaller, but not for the large canals. There are obviously many grave objections to this scheme, as the whole

agriculture of this rainless country is so dependent upon the life-giving stream emanating from them that the water supply cannot be interrupted for any length of time.

Obviously some other method must be devised more in keeping with the agricultural needs of the country.

THE SPREAD OF THE DISEASE IN EGYPT.

Snails of the two genera *Planorbis* and *Bullinus* differ somewhat in their habits.

As a general rule, *Planorbis* is a surface-feeding snail and favours either slowly moving or stagnant and muddy waterways. It feeds on any vegetable material, but for the most part on the rushes and grasses which grow at the margins of the water. Little difficulty is generally experienced in collecting specimens as they occur in masses floating on the surface.

Bullinus, on the other hand, is generally found attached to stones, posts, etc. at some considerable depth below the surface. It is more usually found in clear water such as in large tanks and in more swiftly flowing waterways than is the former. It feeds upon various kinds of aquatic plants which choke these channels in the summer season and may then be found in clusters attached to the under surface of the floating leaves. The main breeding season of *Planorbis* in Egypt would appear to be the spring months, March and April, while *Bullinus* deposits its ova at a later date.

In the case of *Planorbis* the egg-masses are generally deposited upon the shell of another individual of the same species; while *Bullinis* deposits its ova upon stones at the bottom of the canal. The young snails, according to our observations, take about three months to mature.

From our observations the fact emerges that the highest rate of infestation of fresh-water snails occurs round certain centres in the Delta, such as Cairo, Zag-a-zig and Ismailia. These three towns in particular are situated on the main sources of water supply for the Delta and Canal zone, and from them a maximal faecal and urinary contamination of water takes place. Now it has been shown that the range of action of the miracidium is a comparatively limited one.

In the less sparsely inhabited stretches of the fresh-water canal, between these densely populated centres, it is very improbable that the local snails would become infested with miracidia; or if they did, that the cercariae thus produced would be numerous enough to run any chances of encountering a suitable host during their short lifetime.

During the spring months of the year the sluice-gates are open and the Nile water is coursing through the canals of Lower Egypt bearing with it large masses of fresh-water weed and vegetation, and it is to this that most of the infested snails can be found to be clinging. Therefore it would seem that an annual migration of snails to the most distant parts takes place by these means and that this migration corresponds with the breeding season.

From these observations we are led to believe that these towns act as foci in the distribution of bilharziasis to other parts of Egypt.

It would seem possible to greatly minimize the spread of the disease by attaching some kind of mechanical device, such as a wire gauze filtering apparatus, to the lock gates so as to intercept the masses of floating weeds with the contained snails which could then be thrown on to the banks and destroyed. At any rate it would capture many mature and infested snails and thus materially lessen the number capable of carrying the infection.

There is one point as regards the prophylaxis which requires explanation and that is why *S. haematobium* infestations occur so commonly in Egypt, while at the same time it is so difficult to find *Bullinus* infested in any numbers. During the course of routine snail dissections this has been a constant source of speculation.

The highest percentage of infested *Bullinus* we have recorded for one month is 9 per cent.—more commonly it is 1 per cent., or even less; in the same locality the average infestation rate for *Planorbis* with cercariae of *S. mansoni* was 18 per cent., and occasionally reached as high as 54 per cent.

Taking all the year round the *mansoni* infestations in snails were nine times commoner than *haematobium* and add to this the fact that *Bullinus* is much the rarer snail of the two. There is therefore enough scope for further enquiry into this most puzzling aspect of this subject. It may be that there are other more efficient intermediary hosts of *S. haematobium*.

It is important to prevent the entry of infested snails into storage tanks. This can easily be done by guarding the intake with a covering of fine-meshed wire, but it is insufficient to prevent the entry of the minute newly hatched mollusc.

Such tanks utilized in the canal zone during the Egyptian campaign soon became infested with multitudes of *Bullinus* which passed through the gauze-covered intake from the sweet-water canal and which found in this situation a suitable habitat.

Observations showed that snails do not become infested with cercariae till they have reached maturity, that is about the third month, so that there is no danger to be apprehended from such a circumstance, provided that the tanks are frequently cleansed and that necessary measures are taken to guard against any faecal or urinary contamination. Of course their presence in drinking water constitutes a potential danger and is therefore undesirable.

It is already known that in Western Australia certain cases of vesical bilharziasis occurred after the South African war in persons who had never travelled outside Australia. It follows therefore that some Australian molluscs must be capable of acting as intermediate hosts for *S. haematobium* at least.

Under laboratory conditions, the determination of the infectivity of Australian molluscs for both *S. haematobium* and *S. mansoni*, the identification of those species capable of artificial infestation, and the investigation of their life-history, habits, and geographical distribution, are all problems which

urgently require solution. On this knowledge must be based any rational prophylactic measures for the eradication of these potential molluscan intermediaries in Australia and in India as well.

PURIFICATION OF WATER SUPPLIES.

In a country infested with endemic bilharziasis, the presence in a water supply of infested *Planorbis* or *Bullinus*, or even uninfested snails of these species, is sufficient evidence on which to condemn that supply, and to put it out of bounds for troops for drinking, or bathing purposes.

Even when the molluscs have been removed, the water may still harbour cercariae for 36–48 hours. Storage over this period is the most satisfactory method of ensuring its safety. Boiling, naturally, is efficient but impracticable. Certain chemical agents are advocated but they appear less lethal for cercariae than for bacteria.

The commonest bactericide utilized in the Army is bleaching powder—1 part of available chlorine per 1,000,000 parts of water, being considered efficient. In the small book published by the War Office (Memorandum on some Medical Diseases in the Mediterranean War Area), it is stated that one part per 1,000,000 of available chlorine is efficient in killing cercariae. In numerous experiments we have proved the fallaciousness of this statement. Thus, after 2½ hours' immersion in water containing 4 parts per 1,000,000 available chlorine, we have found cercariae alive and very motile. The cercariae were obtained from *Planorbis boissyi*, and the bleaching powder used contained 28 per cent. available chlorine.

One part per million of available chlorine is the maximum that can be added without making the water quite unpalatable. Therefore, unless much greater quantities be added, and some method of dechlorinating afterwards employed, this method of purification of water infested with bilharzial cercariae must prove both unsafe, and unsatisfactory.

PERSONAL PROPHYLAXIS.

This applies especially to Europeans visiting those parts of Egypt in which bilharziasis is endemic.

In the case of troops it is advisable to explain to the men, by means of lectures and demonstrations, the dangers they incur by not obeying the rules drawn up for their guidance. After an ocular demonstration of cercariae in the living *Planorbis*, for instance, they will be much more impressed than by any number of printed notices or orders.

To the sportsman a knowledge of this disease is of the greatest importance. It has been suggested that there is little danger of contracting the disease in the winter season when the snipe and duck most abound, as owing to the low temperatures the snails will not be infested.

From actual observations this does not appear to be the case. The snails once infested remain so for life and attain the maximum degree of infectivity

during the late autumn months. During the early part of the year the rate of infestation appears to be a low one; this circumstance is possibly due to the fact that the larger and more adult molluscs (which are those most generally infested) have been swept away by the winter rains.

There is therefore very considerable risk in contracting bilharziasis by snipe shooting, especially in flooded fields and marshes in the *vicinity of native villages*.

It is necessary to remember that the fresh-water snails are not found in brackish water, nor are they present in swamps which dry up in the summer months, as they cannot survive prolonged desiccation. There are also many large tracts of shooting ground in Egypt, the Fayoum, in which, for some reason or other, neither *Bullinus* nor *Planorbis* occurs.

The sportsman visiting an area infested with bilharziasis should be provided with rubber waders which should reach well above the umbilicus; but it is not sufficient to trust to high boots or thick clothing alone, as the cercariae can easily penetrate their interstices.

There is considerable danger in fishing also; several instances have occurred in Egypt in which soldiers have contracted the infection while thus engaged, apparently from handling newly caught fish in the sweet-water canal. Those addicted to fishing should be warned, on this account, to wear rubber gloves.

Similar precautions must be taken by those whose duties consist in procuring water from canals for bathing purposes. Cases of infection have occurred in men detailed to supply water for baths, horse-troughs, etc.

Finally it must be remembered that there is considerable danger even in riding through infested water; as for instance, in watering horses. Mounted troops in Egypt were apt to be offenders in this respect and constantly had to be warned about it.

Men who conscientiously adopted every other precaution against bilharziasis have been observed "swimming" their horses in a very dangerous locality.

ACKNOWLEDGMENTS.

In completing this Report we should like to place on record our appreciation of the manner in which the investigation has been facilitated and encouraged.

The Laboratory Staff of No. 14 Australian General Hospital—the late Staff-Sergeant C. F. Sullivan, B.Sc., Corporal R. C. Stephens, and Private E. Panelli—have rendered us invaluable assistance and have frequently worked overtime in order to facilitate this work.

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DESCRIPTION OF PLATES III—V.

BILHARZIA CERCARIAE AND ALLIED SPECIES FOUND IN EGYPT.

PLATE III.

Fig. 1. Lateral spined ovum of *S. mansoni*: measurement 0.16 mm. \times 0.06 mm.

Fig. 2. Terminal spined ovum of *S. haematobium*: measurement 0.15 mm. \times 0.056 mm.

Fig. 3. Fork-tailed Cercaria of *S. mansoni*, from liver of *Planorbis boissyi*, showing anterior and ventral suckers, mouth armature, six pairs of poison cells, unpaired glandular structure situated in front of ventral sucker, and posterior pairs of flame cells.

Compiled from average measurements of 40 specimens.

Measurements:

Total length from anterior sucker to fork 0.374 mm.

Body 0.161 mm. \times 0.060 mm.

Antero-posterior measurement of anterior sucker 0.053 mm.

Posterior sucker situated 0.044 mm. from posterior border of body.

Length of tail to fork 0.213 mm. Length of fork 0.066 mm.

Fig. 3 a. Side view of Head of Cercaria of *S. mansoni*, showing appearance of ventral sucker.

Fig. 4. Fork tailed Cercaria of *S. haematobium* from liver of *Bullinus dybowski*, showing internal structure as in 3, with three pairs of poison cells.

Compiled from measurements of 35 specimens.

Total length 0.398 mm. Body 0.190 mm. \times 0.064 mm. Antero-posterior measurement of anterior sucker 0.073 mm.

Posterior sucker 0.049 mm. from posterior border. Length of tail 0.208 mm.

Length of fork 0.081 mm.

Fig. 4 a. Side view of Head of Cercaria of *S. haematobium* showing oval shape of anterior sucker and protrusion of ventral sucker.

Fig. 5. Unpigmented fork tailed Cercaria without eye spots, from liver of *Planorbis mareoticus* from the Zoological Gardens, Cairo. (*Bilharziella*)?

Compiled from measurements of 6 specimens.

Total length 0.296 mm. Body 0.119 mm. \times 0.043 mm.

Tail 0.117 mm. Fork 0.044 mm.

Fig. 6. Sporocyst of 5 from *Planorbis mareoticus*, 0.119 mm. \times 0.102 mm.

Fig. 7. Fork-tailed Cercaria from liver of *Bullinus dybowski* taken in sweet-water canal.

Total length 0.311 mm. Body 0.119 mm. \times 0.031 mm. Tail 0.192 mm.

Fork 0.150 mm. 4 % of snails taken on 2. viii. 17 infected.

Fig. 8. Sporocyst of 7 showing yellow pigmented granules in cyst wall.

Average length of mature sporocyst 0.678 mm. \times 0.075 mm.

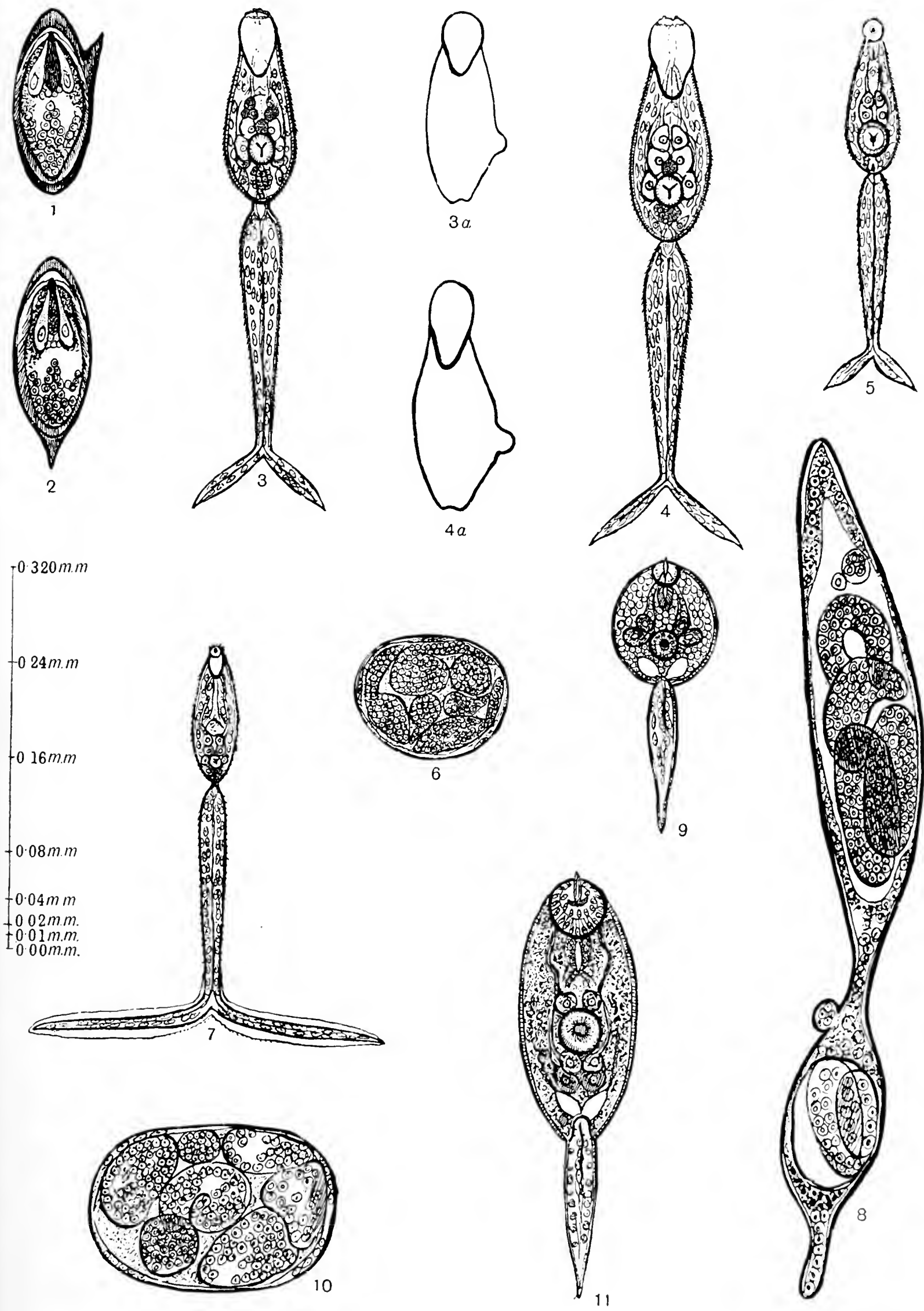
Fig. 9. Cercaria found in 10 % of livers of *Cleopatra bulimoides* described and figured by Loos as *Cercaria cellulosa*, sp. inq. (*Rech. sur la faune parasit. de l'Egypte*, text page 227, Plate XIV figs. 159–161).

Average total length 0.192 mm. Body 0.102 mm. \times 0.06 mm.

Tail 0.09 mm.

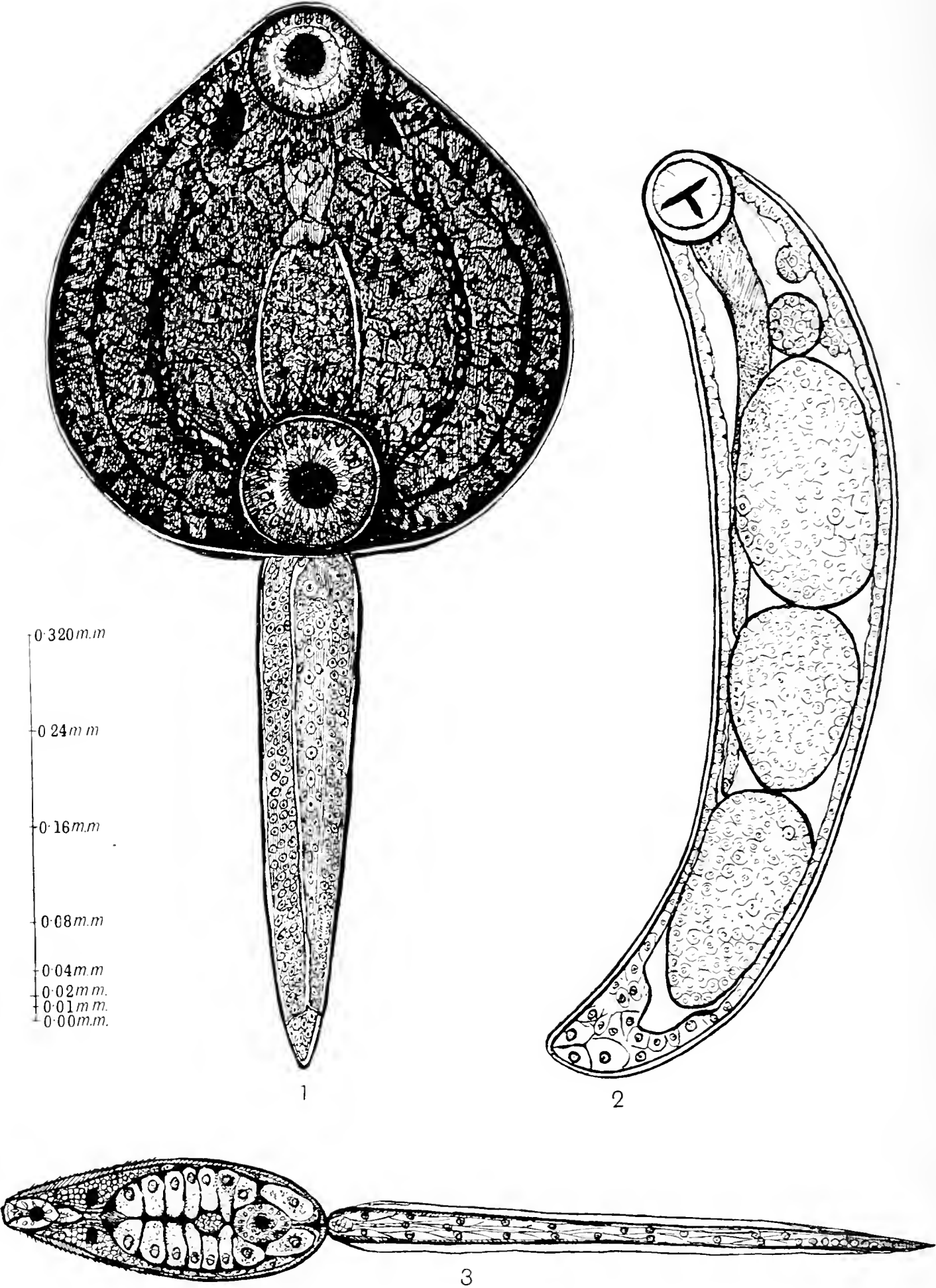
Fig. 10. Sporocyst of 9; Average measurement 0.210 mm. \times 0.13 mm.

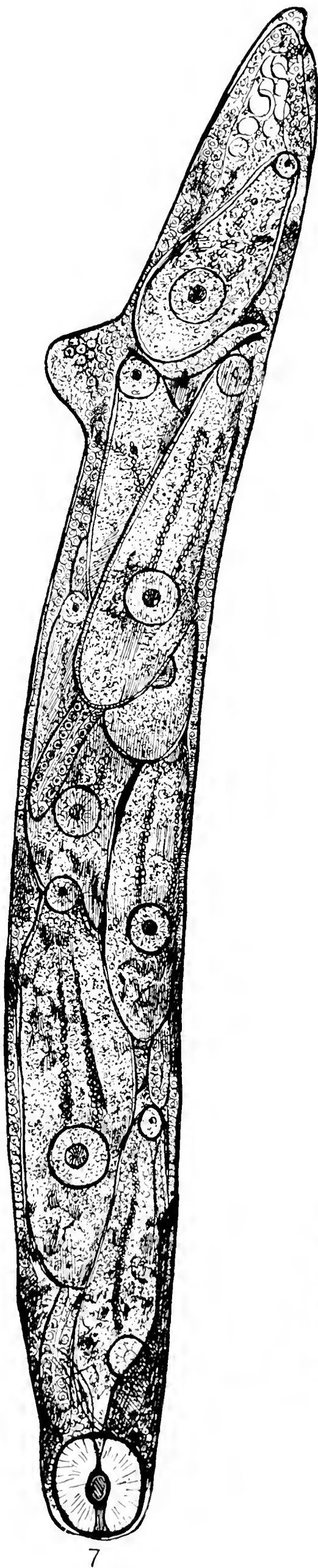
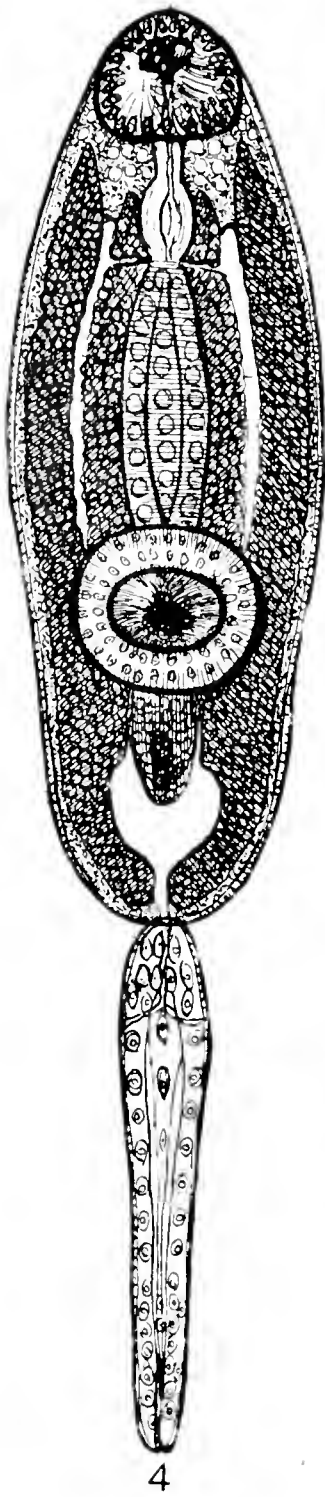
Fig. 11. Cercaria from liver of *Limnaea caillaudi* from the Zoological Gardens, Cairo. (12 % infected.) Total length 0.310 mm. Body 0.195 mm \times 0.086 mm. Tail 0.114 mm. Resembles structure *Cercaria pusilla* described by Loos.



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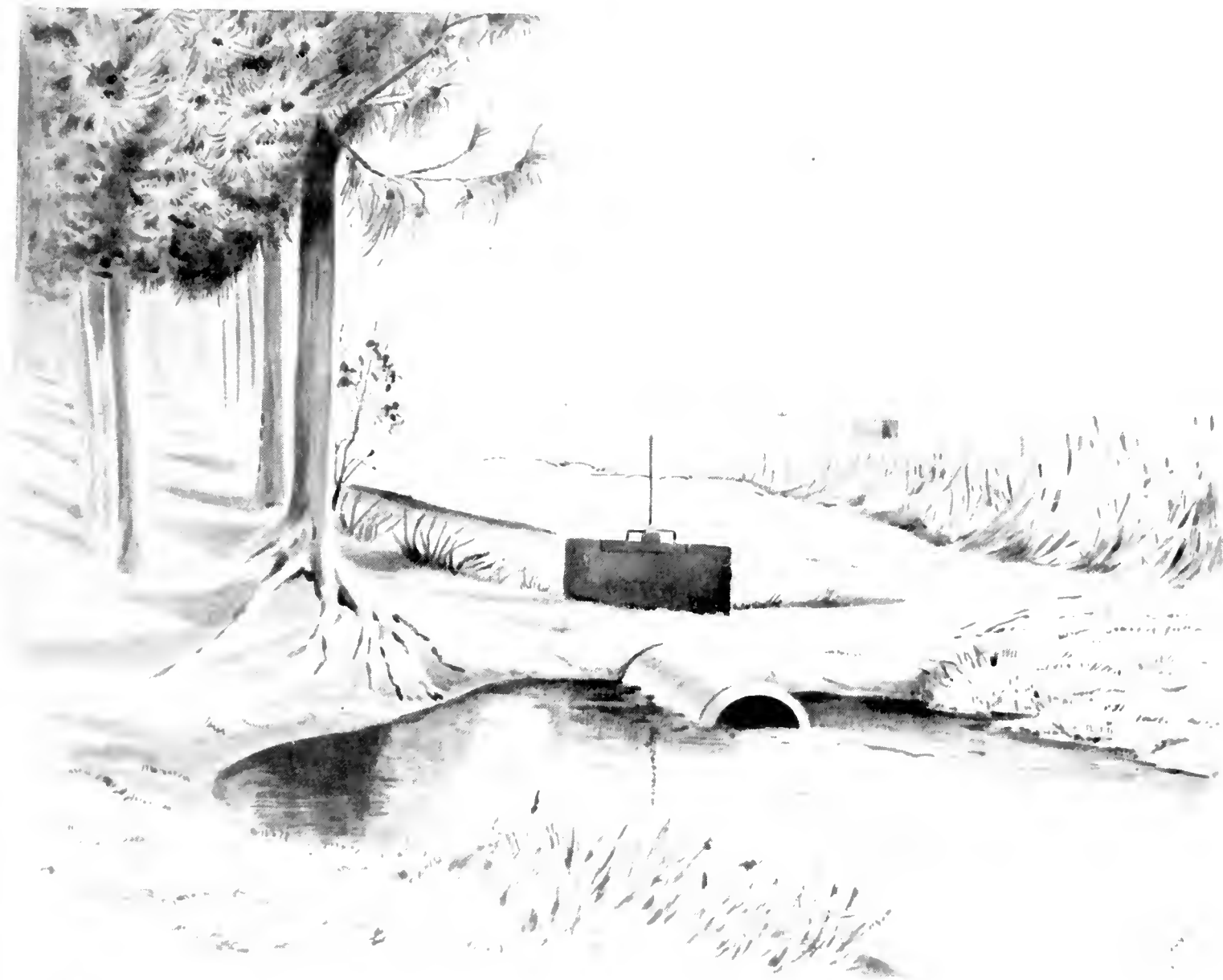
P. H. Manson-Bahr del.

Fig. 1

Transverse section of liver of *Planorbis boissyi* containing large numbers of sporocysts and cercariae of *S. mansoni*, showing the proliferation of the "germ balls" from the sporocyst wall and the development of the cercariae from them. The small amount of normal glandular tissue remaining is well seen. \times ca. 100 diameters.

AL = branch of alimentary canal.

GL = normal glandular tissue.



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Fig. 2

RIFLE RANGE CANAL AT TEL-EL-KEBIR

where troops became infested with *S. mansoni* and *S. haematobium*

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PLATE IV.

Fig. 1. Large Pigmented Cercaria of *Gastrodiscus aegyptius* (Cobbold), *Gastrothylax gregarius* (Looss), or of *Amphistomum conicum* (Rud). The former is a parasite of the horse, the latter two of the buffalo. Looss followed the development of the former, in *Cleopatra bulimoides*, and of the latter two, in his *Physa alexandrina* (Bourg) now known as *Bullinus contortus*. Highly pigmented forms, which he figures as Cercariae of *Amphistomum conicum*, were found associated in the liver of the same snail, with hyaline Cercariae, with pigmented ocular spots, which he describes as Cercariae of *Gastrodiscus aegyptius*.

Total length 0.845 mm. Body 0.474 mm. \times 0.427 mm.

Tail 0.40 mm. Form cysts as in 14.

These Cercariae have been found in *Bullinus contortus*, *Planorbis boissyi*, *Limnaea caillandi*, and *Cleopatra bulimoides*. 1 % *Planorbis*, 5 % *Bullinus* naturally infested.

Fig. 2. Mature redia of 16, showing the anteriorly situated sucker, alimentary canal, germiniferous cells at the posterior end, the numbers of young Cercariae. Size 0.777 mm. \times 0.135 mm.

Fig. 3. *Cercaria pleurolophocerca* (Sonsino) found in the majority of specimens of *Melania tuberculata*, from the Zoological Gardens, Cairo. Very striking Cercaria; tail with cuticular caudal expansion.

Total length 1.0 mm. Body 0.27 mm. \times 0.08 mm. Tail 0.49 mm.

All Cercariae and Rediae measured, and figured in these plates, have been derived from fresh dissections, and were killed in water by heat.

Fig. 4. Cercaria from liver of *Cleopatra bulimoides*.

Total length 0.807 mm. Body 0.5 mm. \times 0.135 mm. Tail 0.300 mm.

(*Cercaria distomatosa*.) Sonsino.

Note the bifid character of the extremity of the tail.

Fig. 5. Cercaria commonly found in *Planorbis*, *Bullinus* and *Physa* (31 % of *Bullinus* naturally infested, and 2 % of *Planorbis*), not described by Looss.

Total length 0.76 mm. Body 0.382 mm \times 0.125 mm. Tail 0.387 mm.

Fig. 6. Encysted Cercaria—same as 13—commonly found in liver of *Bullinus*, especially during the winter months. Average diameter 0.165.

Fig. 7. Orange-pigmented Redia of 13. Length 1.43 mm. \times 0.143 mm., showing muscular terminal sucker, rudimentary alimentary canal, and contained Cercariae in various stages of development, also lateral appendix.

PLATE V.

Figs. 1 and 2. See legend on plate.

HUMAN INTESTINAL PROTOZOAL AND HELMINTHIC INFECTIONS OBSERVED IN MALTA.

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Lieutenant, attached R.A.M.C.

(A REPORT TO THE MEDICAL RESEARCH COMMITTEE.)

THE following observations are the result of two years' examination of the stools of patients invalided to and stationed in Malta. The patients may be roughly divided for convenience into four groups:

- (1) Troops sent straight from the Salonika Hospitals to Malta. This section comprised the bulk of the total number of troops examined, and included a few men from Egypt.
- (2) Garrison Troops—some not patients—including both British and Maltese. With these are included a few British and Maltese Civilians, connected in some way or another with the Army.
- (3) Troops on their way out from England or France. These were mostly Indians and Chinese, who were put off transport at Malta owing to disability.
- (4) Prisoners of War interned in Malta.

Altogether 7323 examinations were made of 3370 individual cases.

¹ The author of this paper, the late Lieut. T. Bentham, returned to England at the beginning of 1919, after spending two years in Malta as Protozoologist attached to the R.A.M.C. During his stay in the Island he made many interesting and new observations upon the protozoal and helminthic infections occurring in British troops, natives, and others. As these observations appeared to us to be well worthy of publication, Lieut. Bentham, at our suggestion, undertook to write a report embodying his findings; but, to our deep sorrow, before its completion he fell a victim to the influenza epidemic which prevailed at the beginning of this year. Nevertheless, his unfinished work was found to contain the greater part of his results; and it seemed to us that, incomplete though it was, it recorded observations which ought to be preserved by publication. We have therefore revised his manuscript, to the best of our ability, and charged ourselves with the responsibility of seeing it through the press, as a tribute to the memory of our friend. We would note, however, that the conclusions and opinions recorded in the paper are those of the author himself, expressed in his own words; and that our editorial activities have been strictly limited to the silent correction of obvious slips and errors in the manuscript, and to the elimination of those ambiguities and repetitions inevitable in a report which was merely an unfinished and unrevised first draft. We are indebted to the author's father, the Rev. T. Bentham, and to his sister, Miss Margaret Bentham, for enabling and helping us to put this work on record.

CLIFFORD DOBELL.
A. C. STEVENSON.

GROUP I.

Group I does not show any features of very great interest. (See Table I.) The percentage of amoebic infections is necessarily very low, because of several important factors which have to be taken into consideration. In the first place an enormous proportion of these patients had suffered from bacillary dysentery in some form or another, and there was no reason to believe that they were otherwise infected. Secondly, and especially towards the end of 1918, a large number of troops had been successfully treated in the Salonika Hospitals, or at least showed a temporary benefit from such treatment. Thirdly, there seems to be, on the part of some medical officers, an insatiate craving for the discovery of animal parasites in every patient. In consequence the stools from patients suffering from such diseases as phthisis, malaria (quinine colitis), influenza, etc., were frequently sent up to the laboratory—some for repeated examination for animal parasites.

Table I.

Troops invalided from Salonika to Malta.

Total No. examined 2485.

Infected with:	No.	%
<i>Entamoeba histolytica</i>	328	13·2
Free Amoebae (not determined)	210	8·5
Other Protozoa	579	27·3
<i>Taenia saginata</i>	1	—
<i>Taenia solium</i>	1	—
<i>Fasciola hepatica</i>	1	—
<i>Trichuris trichiura</i>	8	0·32
<i>Ascaris lumbricoides</i>	9	0·36
<i>Strongyloides stercoralis</i> ...	2	—
<i>Ankylostoma duodenale</i> ...	1	—

In March 1917 an attempt was made to determine the number of amoebic carriers in a certain convalescent camp on the Island. The convalescent dysenterics were confined to a camp of their own, and it was in this camp that a small laboratory was set up for investigation of the stools. Here again the results were somewhat disappointing. There were about 1300 convalescent dysenterics in this camp, but owing to the limited time at our disposal, only 508 cases could be allowed one examination each. This was partly owing to the difficulty of getting the men to provide specimens. Amongst these 508 cases only 52 were found to be carriers of *Entamoeba histolytica*, and there were 64 cases of *Giardia* (= *Lamblia*) infection. This provided the somewhat low percentage of 10·2 for the former. It will be seen from Table I, however, that the total percentage for *E. histolytica* in the Salonika troops is appreciably higher than this, on account of the fact that the majority of individuals were examined more than once each.

In this group worms were of rare occurrence, and the only men of any interest were the two infected with *Strongyloides stercoralis*. One, an officer, had lived in Ceylon and was admitted to hospital for amoebiasis. Examination showed the presence of *S. stercoralis* larvae in large numbers. This officer suffered no ill effects, and on blood examination no appreciable eosinophilia was present.

The other case was a sergeant who was very heavily infected. Extracts from his case sheet may be of interest:

Age 30. Admitted to Hospital from — Battery, to be kept under observation for anaemia. History: Mauritius 1910. Had slight attack of malaria and occasional diarrhoea with blood and mucus at times. Headache, and pain all over body and stomach. Malta 1915. No illness. Khartoum 1916. No illness but severe headaches. At present complains of frontal headache which is continuous, with sudden sharp exacerbations chiefly felt over eye-brows. Tenderness over points of exit of superior and inferior orbital nerves. Suffers from visual troubles manifested by the appearance of zig-zag lines and distortion in shape of objects looked at. Such troubles usually occur during a spasm of pain. Eyesight normal during intervals.

General condition: Thin and anaemic. Left varicocele. No abnormal physical signs in heart, lungs, and alimentary system. Blood count: Red corpuscles 5,920,000. Haemoglobin 45 per cent. Differential leucocyte count: Polymorphs 37 per cent., lymphocytes 30 per cent., large mononuclears 3 per cent., eosinophils 29.5 per cent., and a few mast cells.

In view of the possibility of helminthiasis examination of stool was made, and *Strongyloides stercoralis* and *Entamoeba coli* cysts were found.

Treatment. The patient was instructed to disinfect his hands each time he used pan. He had calomel gr. iv on 14. xii. 17, and gr. v on 15. xii. 17, and the eosinophilia dropped to 14 per cent. Stools swarming with embryos. Thymol treatment as follows: From 18 to 29. xii. 17 Sod. Bicarb. ʒii daily, to clean duodenum of mucus as much as possible. Evening of 28. xii. 17 dose of salts. Nothing in way of nourishment. On 29. xii. 17, at 6 a.m., Thymol 20 grs. in capsules; another dose at 7 a.m. and another at 8 a.m. Dose of salts at 10 a.m.; a little black coffee and tea at 11 a.m. During this time the patient was instructed to lie on his right side, and to abstain from taking any fatty substances all day.

- 5. i. 18. Living embryos still present in stools. Calomel treatment, gr. i t.d.s.
- 10. i. 18. Still on calomel treatment. Living embryos of *Strongyloides* still being passed.
- 15. i. 18. Calomel dose changed. To start with calomel gr. v on alternate days.
- 22. i. 18. General condition not improving: embryos still present. Calomel discontinued.
- 23. i. 18. Sulphur ʒii t.d.s.
- 30. i. 18. *Strongyloides* still present in stools. Off sulphur and put on milk treatment, *i.e.*, absolute milk diet and calomel gr. v on alternate days.
- 11. ii. 18. Embryos still found. Treatment stopped.

GROUP II.

(See Tables II–V.)

This group may be divided into four sections:

- (1) R.A.M.C. and Nursing Staff.
- (2) Other Garrison Troops, including Northumberland Fusiliers, West Yorks, A.S.C. and their relatives.
- (3) Maltese Garrison Troops.
- (4) Civilian employees.

In the second section nothing of importance is to be noted, but the first and third are of no small interest, in that they illustrate the endemic nature of amoebiasis in Malta and its spread among individuals who are more or less compelled to live together under the same conditions and in the same environment.

Table II.

R.A.M.C. Garrison, and Nursing Staff.

Total No. examined 279 (Men 233, Women 46).

Infected with:	No.			%		
	Men	Women	Total	Men	Women	Total
<i>Entamoeba histolytica</i>	33	9	42	14.1	19.2	15
Free Amoebae (not determined)	30	6	36	—	—	12.9
Other Protozoa	107	12	119	—	—	42.6
<i>Oxyuris vermicularis</i>	1	—	—	—	—	—

To illustrate the first section (Table II), the following experiment was made:

N.C.O.'s and men of an R.A.M.C. detachment to the number of 147 were ordered to provide stools for microscopic examination for animal parasites. All these men were of the same company, R.A.M.C., and had been on the Island together for about three years. The members of the Sergeants' Mess, including full corporals, numbered thirty; and of these eleven were found to be infected with *E. histolytica*, giving a percentage of 36.6 on one examination only. Two of the sergeants were on the cooking staff. One of these was found positive during the above routine examination, and the other was negative after repeated examinations, although he was reputed to be a carrier. Subsequently four members of this Mess were admitted to hospital with acute dysentery, although ten members went through an "ambulant" course of treatment with emetine hydrochloride (hypodermically) and emetine bismuth iodide pills. One of these men eventually died of dysentery from a superimposed infection with Shiga's bacillus.

The remaining 117 of this detachment consisted of lance-corporals and privates. Only nine cases, after one examination, were found to be carriers (7.6 per cent.). Two of them afterwards developed acute symptoms though they were all treated by the ambulant method. These 117 men were employed in varied duties, mostly as ward and ablution-room orderlies. The occupations of the nine infected men were as follows—butcher (1), registrar's clerk (1), orderly room clerk (2), ward orderly (3), cook (1), ablution-room orderly (1). The source of infection in four of these cases is fairly obvious, in that the men were employed in close contact with dysentery cases. The registrar's clerk may have obtained his infection through handling case-sheets. Five cooks employed in the same cook-house were infected with *Chilomastix*. Escape from infection by the remaining men is to be attributed to the fact that owing to their duties they probably kept their hands cleaner and were more familiar with the use of antiseptics.

Members of the Nursing Staff, *i.e.*, Sisters, V.A.D.'s, etc., were mostly examined for certificates of health before travelling home through Italy. A few, however, were admitted to hospital with acute symptoms. Some of the carriers developed sub-acute symptoms after the administration of emetine hydrochloride injections and emetine bismuth iodide pills, a condition we have often noticed in the treatment of other cases. The pills sometimes produced violent diarrhoea, with occasional passage of blood and mucus in which no amoebae were present. A similar condition often prevails after treatment with large doses of quinine for malaria.

Forty-six women were examined for infections with *E. histolytica*, and of these nine (19·2 per cent.) were found infected (see Table II).

As will be seen from Table III the remaining Malta Garrison troops gave a very small percentage of infection. They were all admitted with diarrhoea and dysentery, but our records show that this was, in a great number of cases, of bacillary origin.

Table III.

Malta Garrison (1 G.B.N.F., 1 G.B.W. Yorks, R.E., A.S.C., R.A.F.).

Total No. examined 124.

Infected with:	No.	%
<i>Entamoeba histolytica</i>	12	9·6
Free Amoebae (not determined)	22	17·7
Other Protozoa	32	25·0

Twenty-seven of the relatives of these garrison troops were admitted to hospital, and of these 24 were females. These, together with the three males, gave a percentage of infection with *E. histolytica* of 22·2 (see Table IV).

Table IV.

British Civilians, including Relatives of Garrison Troops, Civil Clerks, etc.

Total No. examined 27 (Men 3, Women 24).

Infected with:	No.			%
	Men	Women	Total	
<i>Entamoeba histolytica</i>	1	5	6	22·2
Free Amoebae (not determined)	2	4	6	22·2
Other Protozoa	—	9	9	33·3

Section 3 consists wholly of Maltese Garrison troops, and this was the most heavily infected class with which we had to deal (see Table V), 27·5 per cent. being *E. histolytica* carriers. An example, giving the result of examination of the garrison of a small fort on the coast, will serve to show the prevalence of *E. histolytica* infections among the Maltese. One examination only was made in each case. The garrison of this fort, situated on one of the highest points of the island, numbered 74 men, of whom eight were sergeants and corporals. The remainder were bombardiers and gunners and one trumpeter. Most of

the latter group had their quarters in a large circular underground stone building which was clean and well ventilated. Two men lived at home, and a few others were posted to small outlying forts close to the main buildings. Every week-end a certain number of these men were granted leave to go to their homes. Flies were very prevalent in the summer, especially in and about the outlying smaller forts, but chlorinated water was in general use for drinking purposes.

Table V.

Maltese Garrison Troops (1 and 2 K.O.M.R.M., R.M.A., A.S.C. (M.T.), and R.F.C.).

Total No. examined 200.

Infected with:	No.	%
<i>Entamoeba histolytica</i>	55	27.5
Free Amoebae (not determined)	30	15
Other Protozoa	102	51
<i>Taenia saginata</i>	3	1.5
<i>Trichuris trichiura</i>	13	6.5
<i>Ascaris lumbricoides</i>	2	1

The sergeants and corporals (eight men) had quarters separate from the rest of the garrison. Their ages ranged from 46 to 27½ with an average of 38, and they were older men than the rest of the garrison. All were negative—none of these men being infected with *E. histolytica* as far as the result of one examination was concerned.

Of the remainder (66 men, average age 27), 23 were infected with *E. histolytica*, giving the remarkable percentage of 34.8 on one examination only. Of these men seven had been associated in military life for the previous three years at a fort on the other side of the island. 16 out of the 66 men were free from parasites of any kind, the remaining 50 having parasites, other than *E. histolytica*, in the following numbers: *Chilomastix* 19, *Lamblia* 11, *E. coli* cysts 20, free amoebae 9, *Trichomonas* 2, *E. nana* cysts 4, *Isospora hominis* 1, Helminths 5, Acarina (*Tyroglyphus*) 3. It is therefore not improbable that, if a sufficient number of examinations could have been made, lasting over comparatively long periods of time, nearly 100 per cent. of this garrison would have been found to be infected with *E. histolytica*.

Several of these men were afterwards admitted to hospital with acute symptoms, and the R.A.M.C. orderly who was stationed at the Fort at the time when the examinations were made, told me that, on going his round of inspection, he often observed blood and mucus in latrines. Apparently, in the majority of cases, the Maltese look upon the passage of blood and mucus as a normal event, which can be easily remedied by the administration of some form of tannic acid. The methods of feeding employed by the majority of the town class Maltese leave much to be desired, and large quantities of faecal matter must be ingested together with their food. House-flies are very abundant during the hot weather in Malta; and I have often observed children

with their eyes and mouths black with flies, no attempt being made to drive them away. It is not surprising, therefore, that the Maltese are so heavily infected with all kinds of animal parasites. The comparative frequency of worm-eggs in the stools of this people also serves as an illustration of this condition.

Table VI.

Maltese Civilians, Batmen, Cooks, etc.

Total No. examined 78.

Infected with:	No.	%
<i>Entamoeba histolytica</i>	14	18
Free Amoebae (not determined)	7	9
Other Protozoa	36	46.1
<i>Taenia saginata</i>	1	1.3
<i>Hymenolepis nana</i>	1	1.3
<i>Trichuris trichiura</i>	2	2.5
<i>Ascaris lumbricoides</i>	2	2.5

Section 4 of this Group (see Table VI) comprises Maltese civilians who were, for the most part, prospective cooks and waiters to be employed by the military. These included the servants of one household and of one mess. They were all of a better class than the average Maltese militiaman or gunner, and were not so heavily parasitized. Owing to their occupations, they had better housing, were obliged to be more cleanly in their habits, and lastly, were accustomed to better food. It is no exaggeration to say that *E. histolytica* and worm-eggs were nearly always found in the dirtier and more slovenly people. I think there were three exceptions. 78 individuals were examined with a percentage of *E. histolytica* cases of 18. None of these to my knowledge had suffered from acute symptoms. The servants of the household mentioned above were ten in number (together with three Maltese and two British soldiers included in Table V as garrison cases). On being questioned every one of them denied having had diarrhoea at any time (*sic*). Of these 15 servants, eight were infected with animal parasites, three of them with *E. histolytica*. The occupations of the three were respectively caretaker, messenger, and housemaid.

The servants in the Officers' Mess were seven in number. Four had animal parasites, and only one of them *E. histolytica*.

GROUP III.

This Group (Table VII) consisted mainly of Indian and Chinese troops who were proceeding home from France. The Indians mostly belonged to Labour Corps, and a fair proportion came from the province of Bihar. There were two Burmese included in this section, and a few men from the Naga Hills. The remainder came from various other parts of India. The Chinese consisted of French Colonial Tirailleurs and Labour Corps. Most of them came from Tonkin. In addition to these a few other French Colonial Troops were admitted to Hospital.

Table VII.

Native Labour Corps, etc. (Indians, Chinese, etc.).

Total No. examined 83 (Indians 61, Chinese 18, Somalis 3, Egyptians 1).

Infected with:	Indians	Chinese	Somalis	Egyptians	% (total)
<i>Entamoeba histolytica</i> ...	9	5	—	—	16.9
Free Amoebae (not determined)	4	1	—	—	6.0
Other Protozoa ...	18	—	1	—	22.9
<i>Taenia saginata</i> ...	1	2	—	—	3.6
<i>Hymenolepis nana</i> ...	1	—	—	—	1.2
<i>Clonorchis sinensis</i> ...	—	4	—	—	4.8
<i>Schistosomum haematobium</i> ...	—	—	—	1	1.2
<i>Schistosomum japonicum</i> ...	—	3	—	—	3.6
<i>Trichuris trichiura</i> ...	14	6	—	—	24.0
<i>Ascaris lumbricoides</i> ...	9	4	—	—	15.6
<i>Strongyloides stercoralis</i> ...	—	1	—	—	1.2
<i>Ankylostoma duodenale</i> ...	29	6	—	—	42.2

These men contained a large number of animal parasites, and were, for the most part, infested with worms of various kinds. In all, seven different species of worms were identified from their faeces. The percentage infected with *Ankylostoma* was 42.2 in 83 men examined. The infections with *E. histolytica* gave a fairly low percentage, due no doubt to the comparatively clean methods of feeding adopted by the majority of Indians and Chinese. Yet against this, we have a high percentage of infection with *Trichuris trichiura*, suggesting the faecal contamination of food.

Amongst the findings may be mentioned a case of infection with *Balantidium coli*. The patient, an Indian Christian from Bihar, and belonging to a Labour Corps, was admitted to hospital with coryza and a slight temperature. He had no diarrhoea, but in accordance with custom his stool was sent up with those of the other Indians for the usual examination. It was found to contain *E. histolytica* cysts, *Ankylostoma* eggs, and *Balantidium coli* in large numbers. The patient, who spoke perfect English, was questioned as to whether he had at any time suffered from diarrhoea or dysentery, and answered in the negative. He stated that he had always enjoyed perfect health and had been employed as a labourer in his own country. He had never had anything to do with pigs. Thymol grs. xl was given for the *Ankylostoma* infection, and during the administration of this drug the *Balantidium* temporarily disappeared from the stool, to reappear later, when the treatment was over. Emetine given for the *E. histolytica* infection had no effect upon the balantidia. The patient was eventually discharged cured of his *Ankylostoma* infection, but with the balantidia as numerous as ever.

The case just mentioned belonged to a party of sixteen of the Bihar Labour Corps. All these men were infected with *Ankylostoma* in small numbers and were eventually discharged negative, after the customary dose of forty grains

of thymol. Sometimes the dead worms were found in the stools, usually in a state of decomposition.

The largest infection with *Ascaris* occurred in a labourer from Burma. The eggs of this worm were found in the stool in thousands, and so santonin grs. iii was given. I actually counted out 55 adult round worms, passed from time to time, and the isolation orderlies told me that at least another 50 worms were thrown away in a decomposed condition. All these worms were expelled dead, after a single treatment, and no more eggs appeared in the stool. This man was also infected with *E. histolytica*, *Ankylostoma*, and *T. trichiura*.

Multiple infections were more common among the Chinese, and three examples of this may be given for illustration. These three men were French Colonial Tirailleurs, or Riflemen. Their infections were as follows:

- (1) *Ascaris*, *Ankylostoma*, *Schistosomum japonicum*, *Trichuris trichiura*, *Clonorchis sinensis*, and *Entamoeba histolytica*.
- (2) *Ascaris*, *Ankylostoma*, *Schistosomum japonicum*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Taenia saginata*, and *Entamoeba histolytica*.
- (3) *Ascaris*, *Trichuris trichiura*, *Schistosomum japonicum*, *Ankylostoma*, and *Entamoeba histolytica*.

This last case was that of a Chinaman with cerebro-spinal meningitis and diphtheria.

Autopsy: Body wasted. Excess of fluid at base of brain. A little flaky lymph in region of 4th ventricle and fissure of Sylvius. All superficial vessels congested. Slight excess of fluid in 3rd and lateral ventricles. Choroidal plexus congested. No pleurisy. Both lungs congested and oedematous at their bases. Nothing abnormal found in larynx, trachea, or bronchi. Heart, nothing abnormal discovered. Abdomen: Portal veins and principal branches dissected out. No *Schistosomum japonicum* found. Congestion of mucous membrane of colon, extending to upper part of rectum. Three or four small discrete ulcers, apparently amoebic, in caecum and ascending colon. A fair number of *Ankylostoma duodenale* (worms) recovered from washings of contents of duodenum and jejunum; none found attached to mucous membrane. A large number of ecchymoses found on mucous membrane of duodenum and jejunum. Worms all contained blood. One alive, others dead. A great tangled mass of *Trichuris trichiura* and one *Ankylostoma* were obtained from washings of contents of caecum. No ascarids found. (Patient had been treated, and during life had vomited up four ascarids and passed another twenty *per rectum*.)

Cause of death: Cerebro-spinal Meningitis, Amoebiasis.

Another interesting case is that of an old Chinese labourer, age not ascertained, who was admitted to hospital with "P.U.O." (Pyrexia of unknown origin.) Stool examination disclosed an immense number of *Clonorchis sinensis* eggs and nothing else. Nothing otherwise abnormal was detected, and the man died a fortnight after admission.

Autopsy: Liver small and shrunken, covered all over with small exerescent thickened areas, lighter in colour than the normal liver tissue. There was a large primary carcinoma present the size of an orange. The gall-bladder was full of *Clonorchis sinensis*, and on making a small cut into any part of the surface of the liver, two or three flukes emerged. It was calculated that there were about 5000 of these organisms in the liver tissue alone. The pancreas contained a few flukes and its surrounding mesenteric glands were much enlarged. The flukes which were taken from the gall-bladder were green in colour and contained no blood, but those from the liver were full of blood and fleshy in colour. They measured 14–21 mm. in length by 4–5 mm. in breadth. A large number of the parasites were examined with a view to finding out whether there was also an infection with *Opisthorchis felinus*, but all the worms were found to be *Clonorchis sinensis*. Sections of liver tissue showed carcinomatous thickening of the burrows in which the worms lived, surrounded by a small amount of normal liver tissue. Numerous eggs of this fluke were found in the burrows and in spaces in the new tissue. Unfortunately, only a small piece of the liver was kept, but a fair number of flukes were preserved, some of which are now in the Welleome Bureau, London.

It is to be noted that a large number of these cases suffering from Helminthiasis showed an appreciable eosinophilia but rarely—especially patients who had an *Ankylostoma* infection. It is true that a large number of the men were not very heavily infected, but this blood condition was not evident in the Chinese mentioned above, nor was it noticeable in the Burmese soldier with the large *Ascaris* infection.

The variation of an eosinophil count is well shown by the following table compiled from films made on successive days from the patient, infected with *Strongyloides stercoralis*, already described as a member of Group I (p. 73).

Date	Polymorphs %	Lymphocytes %	Large mononuclears %	Eosinophils %
14. xii. 17	37.5	30	3	29.5
15. xii. 17	30.3	28.7	26.7	14.3
16. xii. 17	42.5	37.5	6.5	13.5
17. xii. 17	40.7	18.3	37.7	8.3
18. xii. 17	51.6	24.6	11.3	12.5
19. xii. 17	41.9	12.7	28	17.4
20. xii. 17	56.7	19.8	7.5	16
21. xii. 17	52.1	19	12.5	16.4
29. xii. 17	26	20	43	11
10. i. 18	31.9	12.4	50.7	5
13. ii. 18	59	20	8	13

In this series of counts there seems to have been a drop in the number of eosinophils after two doses of calomel on 14 and 15. xii. 17, but the count appreciably rises again until thymol was given on 29. xii. 17. It dropped to only 5 per cent. 11 days after this treatment. The other methods of treatment tried during the period from 5. i. 18 to 11. ii. 18—when treatment was stopped—have already been noted on p. 74. During the whole of this time embryos of *Strongyloides* were as numerous as on the first examinations.

GROUP IV.

[The account of this Group, which comprises Prisoners of War interned in Malta, was still unwritten at the time of the Author's death. All the information available is contained in Table VIII.]

Table VIII.

Prisoners of War (German, Turkish, etc.) Internment Camp.

Total No. examined 94 (Germans 68, Turks 20, Greeks 4, Egyptians 2).

Infected with:	Germans	Turks	Greeks	Egyptians	% (total)
<i>Entamoeba histolytica</i>	14	4	—	—	19·1
Free Amoebae (not determined)	12	4	1	—	18·0
Other Protozoa	30	11	1	—	44·7

ON A NEW SACCHAROMYCETE *MONOSPORELLA*
UNICUSPIDATA GEN. N. NOM., N. SP., PARASITIC IN
 THE BODY CAVITY OF A DIPTEROUS LARVA
 (*DASYHELEA OBSCURA* WINNERTZ).

By D. KEILIN, Sc.D.

(From the Quick Laboratory, University of Cambridge.)

(With Three Text-figures.)

THE genus *Monospora* (= *Monosporella* renamed¹) was founded by Metschnikoff in 1884 to designate a parasitic fungus which he discovered in *Daphnia magna*. This monocellular fungus, of which he described only one species, *Monospora bicuspidata*², lives free in the body cavity of its host, where it multiplies actively by budding in a yeast-like manner (Fig. I, 1, 2, 3).

When the body cavity of the host is entirely invaded by the parasites, these grow in size, become elongated, and form club- or sausage-shaped asci in each of which is developed a single needle-like spore having both ends pointed (Fig. I, 4, 5 and 6). When the parasitized host dies, it is filled with ripe spores, and healthy *Daphnias*, which feed on the detritus of their dead and diseased fellows, become infected by ingesting the asci. The latter, when they enter the host's alimentary canal, set free the needle-shaped spores which perforate the gut wall and penetrate in the body cavity (Fig. I, 8) where they germinate laterally, thus starting the new infection (Fig. I, 7).

Metschnikoff's studies on this parasite afford a striking instance of the phenomenon of phagocytosis. In his lectures on inflammation (1893, pp. 83-84), it is stated that directly the spore "appears outside the intestinal wall, it is attacked by leucocytes, which are carried to the spot by the blood-stream. The cells fix themselves on the spore, forming around it a collection of cells, which often fuse together into a plasmodium, which causes the spore to undergo a series of remarkable changes. On being enclosed in the leucocytes the spore

¹ The generic name *Monospora*, given by Metschnikoff to the parasite, is invalidated for the reason stated in the Appendix to this paper, p. 90.

² The species of *Monospora*, discovered by Metschnikoff, was described by him under the name *M. bicuspidata*, and under this name it is referred to in his various publications, nevertheless all the authors I have consulted (Zopf, Hansen, Dangeard, Guillermond, Lafar, Saccardo) wrongly name the species *M. cuspidata* Metschnikoff. I do not know who changed the specific name, but incline to the view that the error may have arisen through a misprint or misquotation, none of the authors mentioned having apparently referred to Metschnikoff's original papers.

first loses its regular contour, becomes sinuous, and finally breaks up into a mass of brownish granules...." By isolating infected *Daphnia*, Metschnikoff succeeded in restoring them to health "thanks to the destruction of the spores by their phagocytes. If on the other hand the phagocytic action is inadequate, owing to the continued increase in the number of spores swallowed or for any other reason, the latter begin to germinate and give rise to budding conidia."

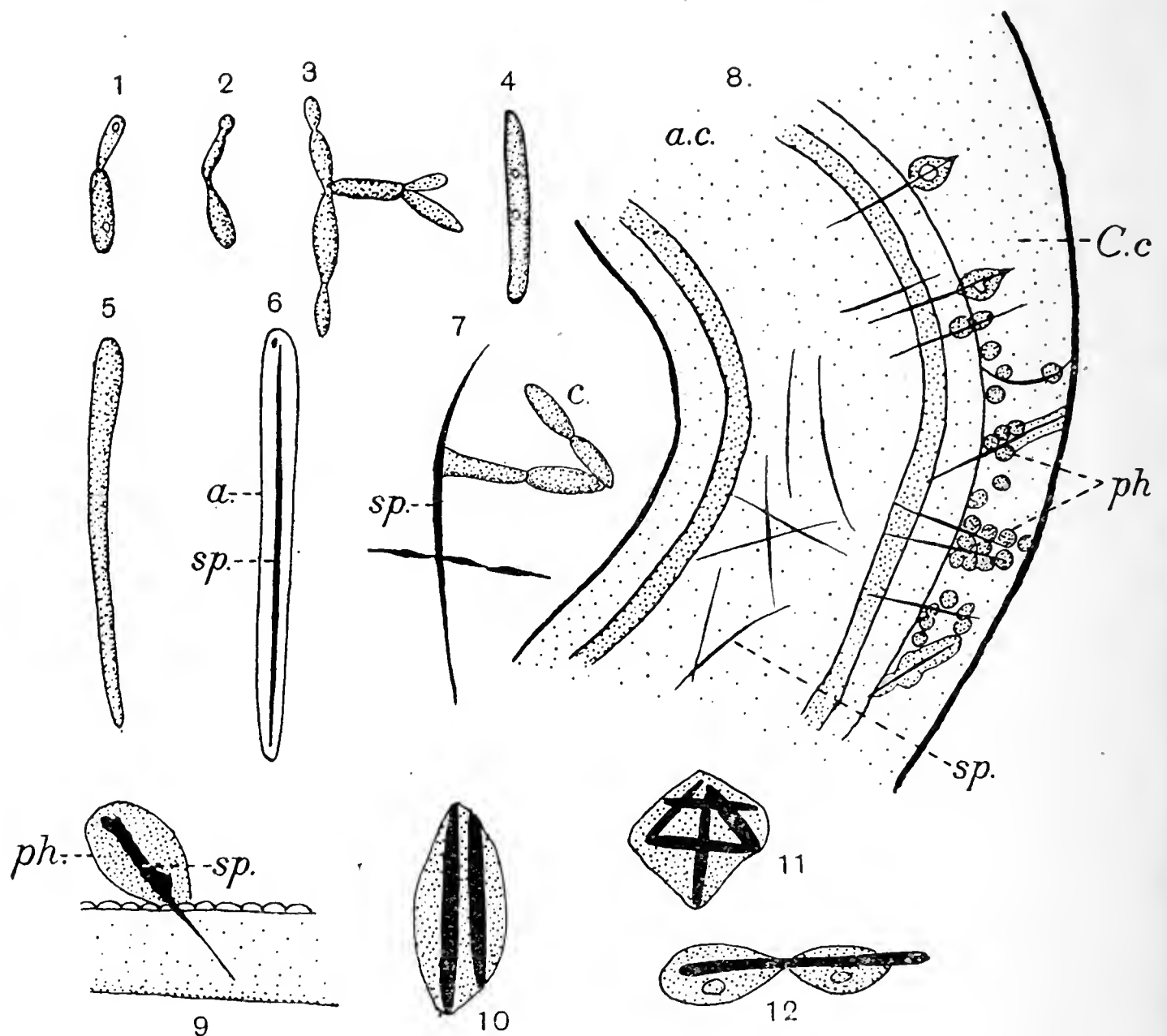


Fig. I. *Monosporella bicuspidata* (Metsch.), after Metschnikoff, slightly schematized. 1, 2 and 3, budding cells; 4 and 5, elongated cells developing into asci; 6, ascus (a.) with spore (sp.); 7, germination (c) of a spore (sp.) that has penetrated into the body cavity of the host; 8, anterior portion of *Daphnia* showing the spores (sp.) of *M. bicuspidata*, free in the alimentary canal (a.c.) or perforating its wall and penetrating into the body cavity (C.c.) where they are surrounded by phagocytes (ph.); 9, spore (sp.) penetrating into the body cavity, partly surrounded and digested by a phagocyte (ph.); 10, phagocyte containing two cells of parasite; 11, plasmodium of phagocytes containing several cells of *Monosporella*; 12, fungus cell surrounded by two phagocytes.

Following upon Metschnikoff, almost all authors (Zopf 1890, Hansen 1904, Dangeard 1907, Guillermond 1907, Lafar 1910 and Saccardo 1911) dealing with *Monospora* refer to Metschnikoff's observation. Chatton (1907), however, seems to be the only author who has actually seen the parasite; thus, in his general account of parasites and commensals living upon Cladocera he mentions (p. 807) that every year during the spring he used to find *Daphnia*

in abundance parasitized by *M. bicuspidata*. His material was derived from a tank at the Jardin des Plantes, Paris.

The genus *Monospora*, or, as we shall call it now, *Monosporella*, has hitherto comprised: (a) the species *bicuspidata* Metschnikoff and (b) a yeast-like fungus found by Bütschli (1876, p. 148, Pl. XIV, fig. 8) in the coelom of a free-living nematode, *Tylenchus pellicidus* Bast. Unfortunately this author's description and figures are insufficient for determining more than the genus to which the parasite belongs.

This summer (1919) I found a new species of *Monosporella*, which I propose to name *Monosporella unicuspidata*, living in a Dipterous larva: *Dasyhelea obscura* Winnertz¹. The larvae of this Ceratopogonid live usually in the thick brown sap which fills the infected wounds of elm or horse-chestnut trees. Whilst larvae collected from the wounds of a horse-chestnut, standing on the grounds behind the School of Agriculture, Cambridge, harboured *Monosporella*, those taken from elms (at Newnham and along the Backs, Cambridge) were not infected by this fungus, they contained however other parasites which will be dealt with separately.

The proportion of larvae infected with *M. unicuspidata* appeared to be low, for but twenty out of several hundred forming the material examined by me were found to be infected. The actual proportion of infected individuals doubtless varies in nature and it must have been greater in this instance. Owing to the larvae being insufficiently transparent to permit the detection of the few parasites that may occur in mild forms of infection, some of these doubtless escaped notice. It appears probable, moreover, that a number of larvae may rid themselves of parasites by phagocytosis as some examples of *Daphnia* do when attacked by few *M. bicuspidata*. The parasitized larvae observed by me belonged to three successive generations of *Dasyhelea* and they were all heavily infected. A parasitized *Dasyhelea* larva is easily recognized by the milky appearance of the body and especially of its posterior segments. Examined microscopically, the larva shows an enormous number of elongated refractive cells, completely filling the body cavity, and in some cases so crowded together that they all take a direction parallel to the long axis of the body of the larva. In spite of the great number of parasites that are present, the larvae are able to move, the fat body seems to be the only organ which is completely destroyed, whereby the larva becomes more transparent and the parasites are better observed. Finally the larva dies and decomposes rapidly, thus setting free the resistant forms of the parasite. In the living larva, even when heavily infected, almost all developmental stages of the parasite are easily seen by cutting open the larva in a drop of normal salt solution or Amman's lactophenol².

¹ The identification of this Ceratopogonid I owe to the kindness of Mr F. W. Edwards of the British Museum.

² Amman's lactophenol, pure or mixed with 0.5 % of cotton-blue, which was used for the examination of the parasite, is highly recommended for similar purposes.

Description of *Monosporella unicuspidata* n. sp. (Fig. II, 1 to 17).

In the young stages, the parasite occurs in the form of small oval cells from 4μ to 10μ long, budding at one end. The buds are usually single (Fig. II, 1 to 6) but occasionally two or three buds are formed simultaneously (Fig. II, 7, 8).

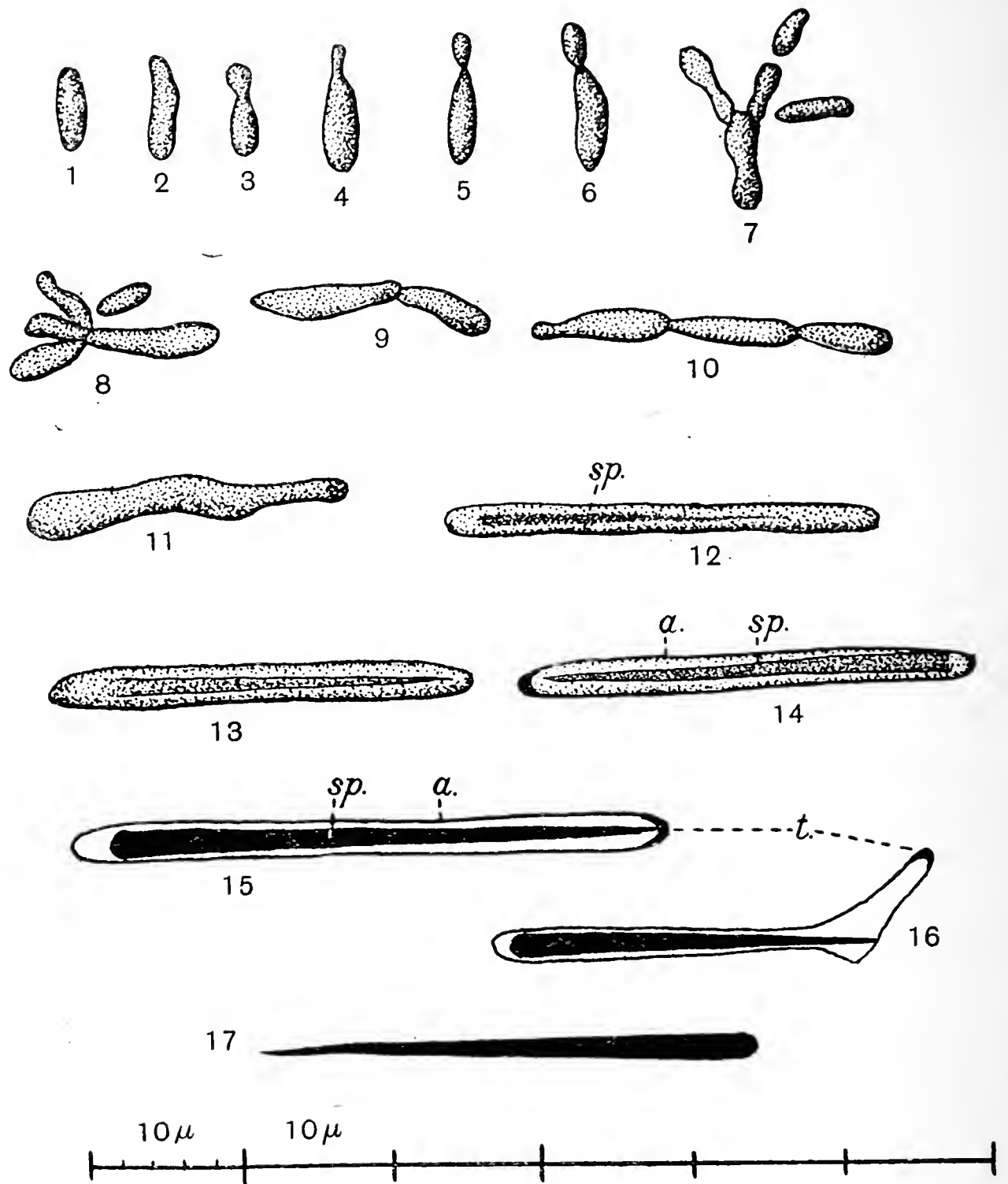


Fig. II. *Monosporella unicuspidata* n. sp. 1 to 6, different stages of budding cells; 7 and 8, rare cases of multiple budding; 9, ordinary budding; 10, chain with three cells; 11, elongated cell developing into ascus; 12, ascus with the beginning of the spore formation (*sp.*); 13 and 14, more advanced stages of spore formation; 15, ascus (*a.*) with well formed spore (*sp.*), *t.* thickened wall of the ascus; 16, deformed ascus; 17, spore. Figures drawn with camera lucida, slightly schematized, the refractory spores being represented in black. The scale of magnification is given beneath the figures.

7, 8). The new buds generally detach themselves very soon and begin to bud; in only a few cases did I find three cells joined to form a chain 24μ in length (Fig. II, 10). When the body of the larva is completely invaded, the parasites become elongated and acquire a uniform shape reaching 30μ in length and

2.5 μ in width (Fig. II, 13, 14). These elongated forms correspond to asci and are at first transparent having finely granulated protoplasm. As development proceeds, the protoplasm near one end of the ascus begins to show a small triangular refractive body which gradually elongates until it occupies almost the whole length of ascus. The refractive body (Fig. II, sp. 14, 15) gradually becomes clearly defined and finally develops into a long needle-shaped unicellular spore with one end sharply pointed, the other end truncated. The space between the spore and walls of the ascus is filled with transparent fluid, and the end of the ascus facing the pointed portion of the spore is thickened. In some cases, after the death of the larva, when the asci escape into the fluid surrounding the insect, the ascus walls become deformed so that the thickened end is bent to one side of the spore (Fig. II, 16). The asci and spores vary in size, the asci measure 30 μ to 40 μ and the spores 24 μ to 35 μ in length; the truncated end of the spore usually measures 1.8 μ across.

I have failed to observe the liberation of the spores from the asci and the germination of spores. In only one larva was the alimentary canal found to contain several free spores (Fig. II, 17), but unfortunately the larva was damaged during examination whereby further observation was precluded.

As the spores of *M. unicuspidata* have only one end pointed, whilst *M. bicuspidata* (Metsch.) has spores with both ends pointed, it is probable that the first named species has a poorer chance of perforating the alimentary canal of its host and this may account for the smaller proportion of infected hosts as compared to what has been observed with *M. bicuspidata* and *Daphnia*.

It is worthy of note that other Dipterous larvae (those of *Rhyphus fenestralis* Scop., *Mycetobia pallipes* Meig., *Aulacogaster rufitarsis* Mcq., *Phaonia cincta* Zett. and a few Eristalines, Drosophilids and Dolichopodids) living under the same conditions as *Dasyhelea obscura*, were not found to be infected with *Monosporella unicuspidata*.

The genus *Monosporella*, hitherto known as *Monospora* Metschnikoff, is often placed by systematists near to the genus *Nematospora* Peglion (Fig. III). The latter, which contains but one species *N. coryli*, discovered and named by Peglion (1901), is a parasite of the hazel-nut in Italy. It is a budding, yeast-like fungus with elongated cells; the ascus is sausage-shaped 65 μ –70 μ long by 6 μ –8 μ broad, and contains 8 spores in two longitudinally disposed bundles of 4, separated by an interval midway along the length of the ascus. These spores are elongate spindle-shaped, with a long flagellum at one end and measure 38 μ –40 μ without the flagellum which is about 35 μ to 40 μ long. Before germination, the spore loses its flagellum and broadens. Peglion succeeded in cultivating *N. coryli*, finding that it grew well on sterilized sugar-beet or meat-broth gelatin and badly in fluid media, where it formed only a mycelium.

The systematic position of the genera *Monosporella* and *Nematospora* is not yet clearly defined. Metschnikoff and Zopf placed *Monosporella* among

the true yeasts, and Peglion divided the *Saccharomycetes* into four genera, i.e. *Saccharomyces*, *Schizosaccharomyces*, *Monospora* (= *Monosporella*) and *Nematospora*.

On the other hand Hansen (1904), to whom we owe the recent classification of *Saccharomycetes* which is accepted by almost all mycologists, considers the last two genera as representing "doubtful *Saccharomycetes*," and he remarks that they are rather rare fungi which were observed only by the authors who discovered them¹, moreover, that he and his collaborators had searched for them in vain in the hope of obtaining material for purposes of study. Notwithstanding the great authority of Hansen, I cannot but

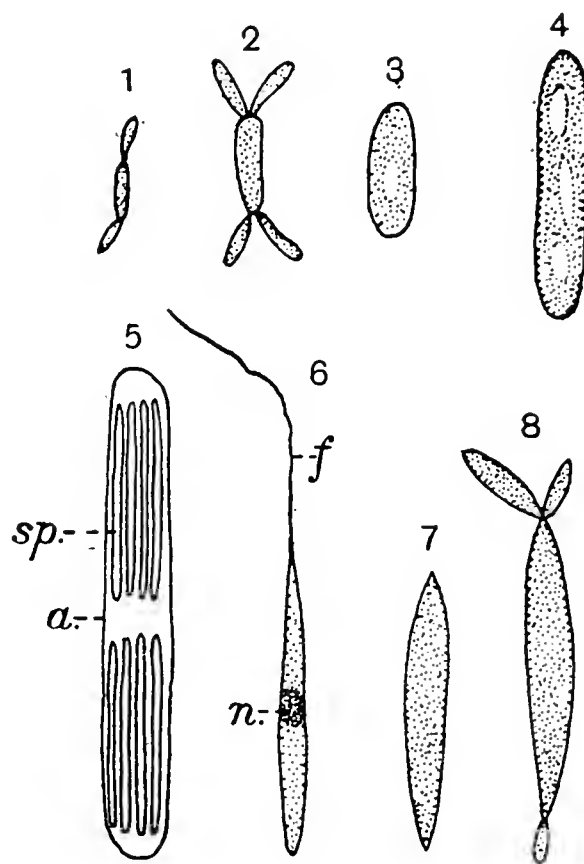


Fig. III. *Nematospora coryli* Pegl. after Peglion. 1 and 2, budding cells; 3–4, stages of growing cell; 5, ascus (a.) with eight spores (sp.); 6, spore stained with gentian violet, showing the nucleus (n.) and flagellum (f.); 7, spore after losing its flagellum; 8, budding spore.

believe that *Monosporella* and *Nematospora* have been sufficiently known for purposes of classification, and that they should without hesitation be placed in the family of *Saccharomycetaceae*. In fact all the characters of this family, as defined by Hansen himself, cover perfectly well the representatives of these two genera. Thus Hansen's classification, with the few modifications and details added by Lafar (1910), is as follows:

I shall omit the expressions (for the group A) "true *Saccharomycetes*" and (B) "doubtful *Saccharomycetes*" and shall add more details bearing on the genus *Monosporella*. In Hansen's classification the *Saccharomycetaceae* are widely separated from the genus *Schizosaccharomyces* for which he creates the family *Schizosaccharomycetaceae*.

¹ I would note that *M. bicuspidata* Metsch. was often observed by Chatton (1907) and cultures of *N. coryli* were sent by Peglion to the late Prof. A. Giard of Paris who tried to inoculate hazelnuts with the fungus.

Family **Saccharomycetaceae**. Monocellular, sporogenic budding fungi. Each cell is a potential sporogenic cell (or ascus). The number of spores in the ascus is usually 1 to 4, seldom 12. The spores are monocellular. Typical mycelium is formed by a few species.

A. Spore rounded, oval, pileate or lemon-shaped with or without projecting rim.

I. GROUP. In Saccharine nutrient liquids they furnish only sedimental yeast at the outset, surface films occur only at a later period, if at all. Films more or less mucilaginous. Spore smooth, globular or oval, with 1 or 2 membranes. Spore germinates either by gemmation or by producing a promycelium. Great majority produce alcoholic fermentation.

Genus 1. *Saccharomyces* Meyen. Spore with single membrane; germinates by ordinary gemmation. In addition to yeast cells a few of them produce mycelium with well defined septa.

Genus 2. *Zygosaccharomyces* Barker. Ascus is formed after cell fusion.

Genus 3. *Saccharomycodes* Hansen. Spore with a single membrane, germinates into a promycelium, the new cells, being incompletely separated, form a mycelium with well defined septa.

Genus 4. *Saccharomycopsis* Schiønning. Spores with two membranes.

II. GROUP. Film produced on the surface of nutrient solution immediately after the same has been inoculated. The film has a dry dull appearance due to the inclusion of air bubbles. Several species produce esters, and a few of them do not cause fermentation. Spore with a single membrane, of different shapes with or without projecting edge.

Genus 5. *Pichia* Hansen. Spore rounded, hemispherical or irregular and angular. A strong mycelium is formed. No fermentation.

Genus 6. *Willia* Hansen. Spore pileate, or lemon-shaped, with a projecting rim. Most of the species possess considerable ester-forming powers, but a few do not produce fermentation.

B. Spores acicular or spindle-shaped, parasitic fungi.

Genus 7. *Nematospora* Peglion. Elongated budding cells; sausage-like ascus containing eight unicellular spores in two bundles of four. Spore elongated, spindle-shaped with a long flagellum, which is lost before germination. Parasitic on hazel-nuts in Italy. Grows well on sugar-beet or meat-broth gelatin, badly in liquids where it forms mycelium only. One species: *N. coryli* Peglion (Fig. III).

Genus 8. *Monosporella* (= *Monospora* Metschnikoff 1884). Budding, yeast-like fungi, each cell a potential ascus. The latter is elongated and

produces one unicellular acicular spore. Parasite in the blood of invertebrates. Not yet cultivated. The genus comprises the following species:

(1) *M. bicuspidata* (Metschnikoff 1884). Asci slightly narrowing at one end; spores pointed at both ends. Parasite in the body cavity of *Daphnia magna* (Crustacea) (Fig. I).

(2) *M. unicuspidata* n. sp. Asci of elongated but regular form with the wall thickened at one pole. Spore pointed at one end, truncated at the other. Parasite in the body cavity of Ceratopogonid larvae: *Dasyhelea obscura* Winnertz. (Insecta: Diptera.) (Fig. II.)

(3) *M. sp.* Yeast-like fungi found by Bütschli (1876) in the coelom of a free-living nematode: *Tylenchus pellicidus* Bast. (Vermes.)

(4) *M. (?) sp.* Yeast-like fungi of elongated shape found by Caullery and Mesnil (1899 and 1911) in a Polychaete worm *Potamilla torelli*, where they seem to produce a special kind of tumour ("néoformation papilloma-teuse"). They consider this yeast to be related to *Monospora* although they did not succeed in finding the spores. They also mention a similar yeast occurring in a pelagic Copepod *Acartia*.

Family **Schizosaccharomycetaceae**. Endosporogenic, monocellular fungi, reproduce by fission which is preceded by the formation of a septum that at once commences to divide into two lamellae from outside. No budding occurs. Spores unicellular of which 1-8 occur in each ascus. In some cases formation of asci is preceded by fusion. Spores stained blue by a solution of iodine in potassium iodide. The cells never contain glycogen (a contrast to *Saccharomycetaceae*). Produce alcoholic fermentation; one genus *Schizosaccharomyces* Lindner.

Acknowledgment.

I am much indebted to Professor G. H. F. Nuttall for valuable suggestions in connection with this study.

APPENDIX.

Concerning the re-naming of Metschnikoff's genus Monospora.

According to Lafar (1910, p. 292), "the genus *Monospora* Metschnikoff ought really to be re-named, since this title has already been applied by Hochstetter, to one of the Flacourtiaceae."

This statement is correct. I would add that Hochstetter's name dates from 1841. However, in Warburg's monograph of the Flacourtiaceae (1894, in Engler and Prantl's *Die natürlichen Pflanzenfamilien*, Part III, p. 37) I find that the name *Monospora* Hochstetter is condemned as a synonym of *Trimeria* Harvey (1838).

On the other hand I find that the name *Monospora* was also given by Solier in 1845 to an Alga of the family Rhodomelaceae; the name was accepted by all specialists of the group and is actually quoted in botanical text-books.

The foregoing evidence serves unfortunately to condemn Metschnikoff's name *Monospora* as being preoccupied. This is regrettable because the name has long been associated with that author's early observations on the important phenomenon to which he gave the name of phagocytosis. I have endeavoured, however, to replace the original name by another which expresses the same meaning, and *Monosporella*, which I propose, seems most fitting, other suitable names being preoccupied.

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ON THE OCCURRENCE OF A SUPPLEMENTARY
CHROMATIC BODY IN *MAUPASELLA NOVA* CÉPÈDE
(CILIATA ASTOMA), AN INTESTINAL PARASITE OF
EARTH-WORMS (*ALLOLOBOPHORA CALIGINOSA*
SAVIGNY).

BY D. KEILIN, Sc.D.

(From the Quick Laboratory, University of Cambridge.)

(With Plate VI.)

Maupasella nova Cépède was discovered by Maupas (1877) in the alimentary canal of an Algerian earth-worm and subsequently described by Cépède (1910). As was well remarked by the latter author, this ciliate varies much in size and in form, long specimens measuring 80–95 by 25μ , and short ones only 50–75 by $27\text{--}47\mu$. The essential characters of *Maupasella* are: (1) the presence of an anterior fixing apparatus in the form of a conical process derived from thickened ectoplasm, (2) an elongated ribbon-like macronucleus, (3) irregularly disposed contractile vacuoles, and (4) dense ciliation. Moreover, the micronucleus (Pl. VI, fig. 18 and *m* in all the other figures), which is often difficult to see, is spindle-shaped, with its axis parallel to that of the ciliate's body, strangulated in the middle and with the chromatin condensed into a disc lying in the strangulated portion of the spindle.

In many specimens of *M. nova* obtained from the alimentary canal of *Allolobophora caliginosa* Sav. collected near Paris (France), I found a ribbon-like supplementary chromatic body which I propose to describe here. These bodies occur in specimens of *Maupasella* which are of different size and form. They stain deeply and uniformly with basic stains, especially with iron-hematoxylin, and never show the granulated structure which is so characteristic of the macronucleus. Their position in the endoplasm of the ciliate, as well as their shape, varies very much (Pl. VI, figs. 1–17); in some specimens of *Maupasella* the chromatic body is straight and parallel to the macronucleus, while in others it is curved in the form of an S or C, or is strangulated in the middle and having one end twisted round the macronucleus (Pl. VI, figs. 9, 10 and 12), but in no case has any continuity between the latter and the chromatic body been observed. In very elongated examples of *Maupasella*, which are going to divide (Pl. VI, figs. 9 and 10), and in already dividing specimens

(figs. 11, 12, 13, 14 and 17), the chromatic body always lies in the posterior portion of the ciliate, so that after the division, this body is inherited by the posterior individual, while the anterior is always devoid of it. This shows that the chromatic bodies of different specimens of *Maupasella* are in no way related to each other, but are formed independently in each ciliate, which possesses them.

In spite of a great number of *Maupasella* having been found to contain the chromatic body, I have not yet been able to trace its origin and all I can do at present is to discuss critically several explanations which might be offered regarding its nature.

(1) The absence of any structure or stages of reproduction in this body, as well as its great polymorphism, render very doubtful the supposition that it represents an unknown parasite of ciliates.

(2) It might be supposed that it is derived from the macronucleus of the ciliate by longitudinal division. The occurrence of a double macronucleus is already known in ciliates of the family Anoplophryinae. It was first described by Schneider (1892) in *Hoplitophrya* sp., an intestinal parasite of a fresh-water Oligochaete, among several specimens of which he found 3 or 4 individuals with two macronuclei. These abnormal ciliates showed an ordinary multiple or catenular process of segmentation during which both macronuclei underwent a similar division.

Double macronuclei were also observed by Léger and Duboscq (1904, p. 343) in *Anoplophrya brasili*, an intestinal parasite which they discovered in *Audouinia tentaculata* Mont. As to the origin of the two parallel macronuclei, they do not agree with Schneider (1892), who supposed them to arise from an abnormal conjugation: they explain them, on the other hand, as a result of an ordinary longitudinal fission of a single macronucleus.

But even if we admit the occurrence of longitudinal cleavage of the macronucleus in Anoplophryinae, this process will not explain the origin of the supplementary chromatic body in *M. nova*, for the following reasons: (a) the form and structure of the chromatic body differ very much from those of the macronucleus, (b) there is no continuity between these two structures, and (c) the chromatic body never undergoes segmentation when the macronucleus divides.

(3) The chromatic body does not originate from the micronucleus, as in almost all specimens of *Maupasella* which contained it, the micronucleus was normally developed.

(4) Its origin cannot be interpreted as a result of abnormal conjugation, as this process of reproduction has not yet been observed in Anoplophryid parasites of earth-worms.

(5) It could be suggested also that this body is related to the so-called spicule, an endoplasmic rigid body which normally exists in other Anoplophryinae parasitic in earth-worms, namely *Mesnilella secans* Stein, *M. clavata* Leidy, *M. spiculata* Warpachowsky and *M. fastigata* Möbius. Unfortunately

we know very little of the structure and development of the spicule, which according to Möbius (1888, p. 104) stains well with osmic acid and safranin.

(6) According to Fauret-Frémiet (1907, quoted by Cépède, 1910, p. 500) the endoplasm of *Anoplophrya striata* contains mitochondrial bodies. The existence of extranuclear chromatin was, on the other hand, mentioned by Cépède in *Anoplophrya alluri* and *Herpetophrya astoma* Siedlecki. The endoplasm of *M. nova* undoubtedly contains diffused chromatin as it stains usually with basic stains. Very often the extranuclear chromatin is more condensed into two lateral bands parallel to the macronucleus (Pl. VI, figs. 8, 10, 11 and 14). It is possible that the supplementary chromatic body is the result of a further stage of condensation of the extranuclear chromatin, produced under the influence of some change in the external conditions of life of the ciliate, in this case, in the alimentary canal of the host.

I do not think that a similar supplementary chromatic body has yet been described in other ciliates, and I hope that further observations on similar abnormalities in other parasitic or free-living species will help to elucidate the origin and the nature of this structure.

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EXPLANATION OF PLATE VI.

MAUPASELLA NOVA CÉPÈDE.

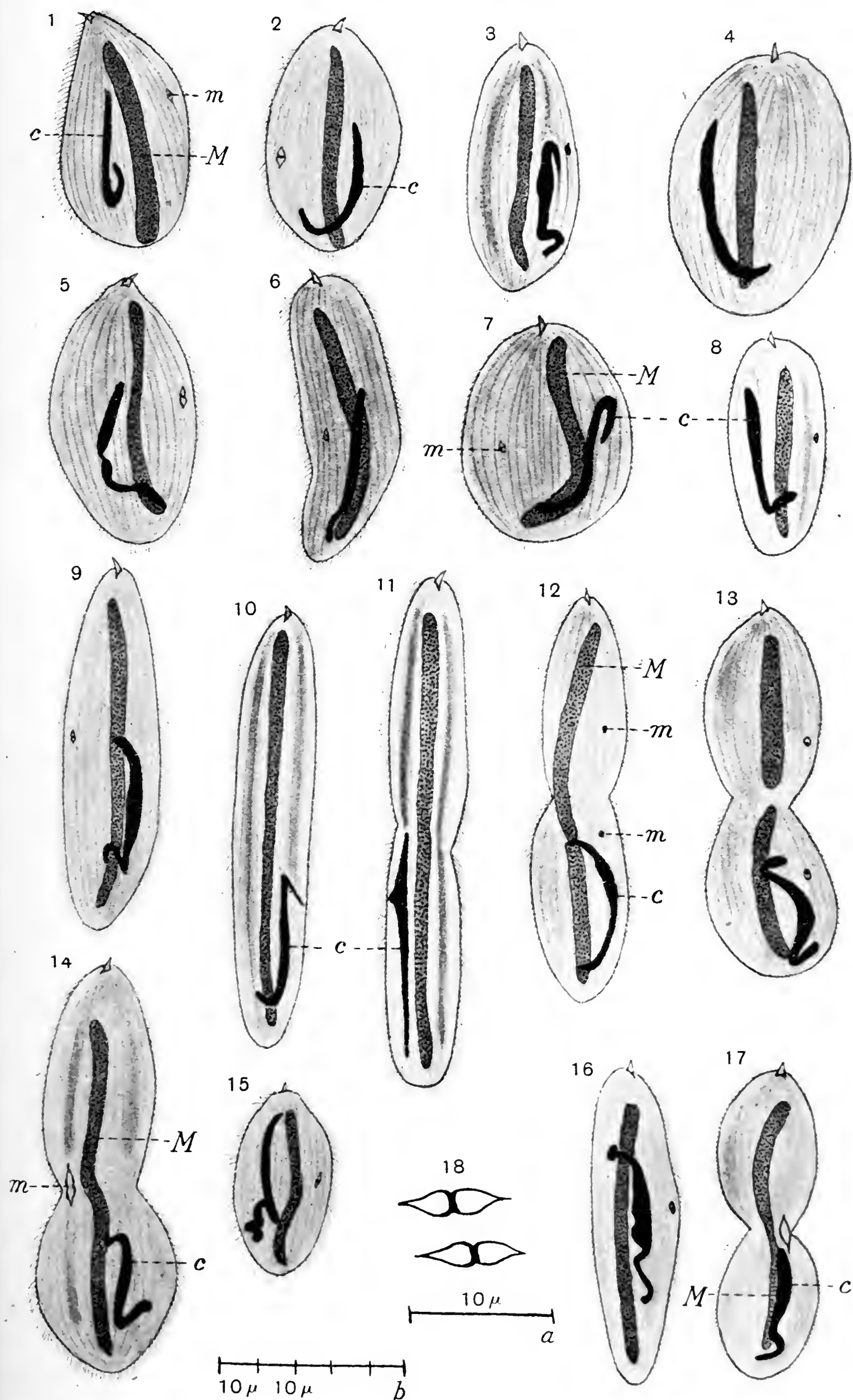
c. supplementary chromatic body; *M.* macronucleus; *m.* micronucleus. *a.* scale of magnification for all the figures except fig. 18; *b.* scale for fig. 18.

Figs. 1-9 and 15, 16. Different forms of the supplementary chromatic body in *M. nova*.

Fig. 10. Elongated form of *M. nova* showing the chromatic body occupying the posterior portion of the ciliate.

Figs. 11-14 and 17. Dividing stages of *M. nova* with the supplementary chromatic body localized in the posterior individual.

Fig. 18. Micronuclei of *M. nova*.



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INTESTINAL HELMINTHS IN INDIANS IN
MESOPOTAMIA.

BY CHARLES L. BOULENGER, M.A., D.Sc.
(Reader in Helminthology, University of Birmingham.)

WHILST engaged on protozoological investigations of dysentery and allied diseases in Mesopotamia I had the opportunity of examining the stools of a large number of hospital patients, both British and Indian, and of noting the presence of worm ova as well as protozoan parasites.

The British patients proved to be almost entirely free from helminth infection; on the other hand, as was to be expected, such parasites were of frequent occurrence in the Indians and the object of this note is to give a brief account of the incidence of the different forms met with.

The stool examinations were made primarily for protozoan parasites, the samples were not sedimented or centrifuged and at the most two smears were examined for each case. According to Clayton Lane (1916), to establish the absence of worm infection at least fifteen smears must be examined from each faecal sample; it is obvious therefore that the percentages obtained by me for the different worms are too low, probably about half those which would have been found had the proper helminthological technique been employed. The figures are however, in my opinion, worth publication, since very few data of this kind are available, moreover they afford me an opportunity of calling particular attention to certain worms observed.

The total number of individuals examined was 1180, these consisted chiefly of dysentery cases and patients convalescent from that disease, a number suffering from other complaints (*e.g.* anaemia) are however included, as well as 200 healthy men, selected as "controls" in various investigations.

The findings are shown in the following table:

Number of individuals examined 1180				
<i>Taenia saginata</i>	14	=	1.2	%
<i>Hymenolepis nana</i>	23	=	2	„
<i>Ascaris lumbricoides</i>	62	=	5.2	„
<i>Oxyuris vermicularis</i>	1	=	0.08	„
<i>Ancylostoma duodenale</i>)	219	=	18.5	„
<i>Necator americanus</i>)				
<i>Trichostrongylus</i> sp.	14	=	1.2	„
<i>Strongyloides stercoralis</i>	6	=	0.5	„
<i>Trichuris trichiurus</i>	59	=	5	„

The percentages for some of the commoner parasites, *e.g.* *Ascaris lumbricoides*, seem very low; this is due, partly, as explained above, to the technique employed and partly, no doubt, to the fact that the majority of the individuals examined were hospital patients who had already undergone some form of treatment. Even after allowance is made for these factors the infections are not heavy as compared with those of natives examined in India (cf. Clayton Lane, 1916); a similar series of Turkish prisoners of war examined by precisely the same methods gave me a percentage of 22·2 for *Ascaris*, *i.e.* more than four times that in Indians in Mesopotamia.

The two hookworms are considered together, since the eggs of *Agchylostoma* and *Necator* are difficult to distinguish with any certainty unless large series of measurements are made, both worms were however frequently recovered after the administration of vermifuges. *Necator* seemed rather more common than *Agchylostoma* and my records of the egg measurements show the relative abundance of the two forms to be as 4:3.

The eggs identified as those of *Trichostrongylus* measured 80–99 microns in length and when passed in the faeces were in a late “morula” stage, *i.e.* in a much later stage of development than those of the hookworms; they resembled in every respect the eggs of species of *Trichostrongylus* with which I was familiar in domestic animals. The extreme measurements of the ova are slightly in excess of those given by various authors for the different species of this genus, the latter measurements are however seemingly based on eggs taken from the uteri. I have frequently noted in various Strongyles that measurements of ova from faeces are often greater than those of ova taken from the maternal tissues.

The parent worms were unfortunately never recovered after the administration of vermifuges, in spite of several attempts.

Trichostrongylus ova were also frequently observed in the faeces of Kurdish and Persian coolies employed in various labour corps in Mesopotamia.

Until recently three species of *Trichostrongylus* (*T. colubriiformis*, *T. vitrinus* and *T. probolurus*) had been recorded by Looss (1905) as occasional parasites of man in Egypt; in 1914, however, Jimbo described a fourth species, *T. orientalis*, found in a large percentage of cadavers examined by him in Japan; as suggested by Ransom (1916) the various species of this genus are probably much commoner parasites of man than is generally supposed and their distribution in man will no doubt be greatly extended as increased emphasis is laid upon laboratory methods in medical diagnosis.

Hymenolepis nana is another parasite to which I desire to call particular attention. As shown in the table, it was the commonest Tapeworm met with in Indians. The occurrence of this species is, I understand, well known to many medical men working in India, it is a fact however which for some reason or other has not found its way into the text-books on Parasitology and Tropical Medicine; even in the more recent editions and the newer works (*e.g.* Fantham, Stephens and Theobald, 1916) the distribution of this parasite

is constantly given as: parts of Europe, N. and S. America, Egypt, Siam, Japan, and the Philippines.

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ON SOME NEMATODE PARASITES OF THE ZEBRA.

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(With 7 Text-figures.)

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INTRODUCTION.

HAVING during the last few years been particularly interested in the Nematode parasites of horses and donkeys I was very pleased to find a tube containing Strongyles from the zebra among some material recently forwarded to me by Professor G. H. F. Nuttall, F.R.S. These worms formed part of a collection of Helminths from Nairobi, British East Africa, which had been sent for identification by Mr R. Eustace Montgomery, Director of the Nairobi Veterinary Pathological Laboratory.

The specimens were in a good state of preservation and, although not numerous, they included representatives of eight species, three hitherto undescribed, one of which is taken as the type of a new genus. The specific identity of the host was unfortunately not ascertained, the worms being labelled “Zebra, R. Ruaraka.”

As pointed out by Leiper (1911), the Nematodes parasitic in the zebra consist of a number of species occurring in the domestic equines as well as others peculiar to itself, of the latter among the bursate forms perhaps the most interesting are those included by that authority in the genus *Cylindropharynx*. Both the known species of this genus are to be found in Mr Montgomery's collection, and, whilst describing some new forms of *Cylicostominae* in this paper, I take the opportunity of somewhat amplifying Leiper's descriptions of these species.

The worms from the Nairobi material have been identified as follows:

1. *Strongylus vulgaris* (Looss, 1900).
2. *Cylindropharynx brevicauda* Leiper, 1911.
3. „ *longicauda* Leiper, 1911.

4. *Cylicostomum minutum* Yorke and Macfie, 1918.
5. „ *zebrae* sp. n.
6. „ *Montgomeryi* sp. n.
7. *Triodontophorus serratus* (Looss, 1900).
8. *Craterostomum tenuicauda* gen. et sp. nn.

The species numbered 1, 4 and 7 are also parasites of the domestic equines.

Family STRONGYLIDAE.

Subfamily CYLICOSTOMINAE Railliet, 1914.

Genus CYLINDROPHARYNX Leiper, 1911.

Cylindropharynx brevicauda Leiper, 1911.

The type species of the genus is more abundantly represented in Mr Montgomery's collection than *C. longicauda*. Leiper has given a fairly complete account of the species, a few characters are, however, omitted from his description and I take this opportunity of adding to our knowledge of certain important structures, *e.g.* the oral leaf-crowns and the external genitalia in the male sex.

The specimens before me measure: Females, 5.6–8 mm., males, 5–7.3 mm. in length; maximum breadth of the body, about 0.45 mm.

The head (measured at the anterior extremity of the mouth-capsule) has a diameter of 0.12–0.15 mm. The mouth is circular and surrounded by a mouth-collar which appears almost semicircular in a lateral view (Text-fig. 1).

The lateral papillae are not prominent, they are situated in a slight depression of the oral margin and have trifurcated extremities, recalling the similar structures in *Oesophagodontus robustus* (cf. Boulenger, 1916). The submedian papillae are conspicuous, each consists of a leaf-shaped appendage carried on a cylindrical base (Text-fig. 1, A).

The anterior leaf-crown consists of six triangular elements unequal in size and arranged in a very characteristic manner; four occupy a submedian position and are of equal, relatively small size, the two remaining leaves are much broader and situated laterally, each is usually notched at the apex and longitudinally grooved, suggesting an origin by fusion of at least two elements. Owing to the lack of radial symmetry of these organs, the head presents very different appearances when viewed laterally or from the dorsal or ventral sides (Text-fig. 1, A and B).

The posterior leaf-crown consists of a circle of twelve broad, elongated leaves arising from the anterior margin of the mouth-capsule, the leaves are extremely thick, in profile (Text-fig. 1, A) they might be taken for anteriorly directed branches of the oral capsule.

The very characteristic mouth-capsule measures 0.3–0.4 mm. in length with a maximum diameter of 0.09–0.12 mm., its internal surface is lined with a transparent chitinous layer, as in many species of *Cylicostomum*.

A small oesophageal funnel occupies the anterior cavity of the oesophagus, the latter being almost cylindrical in shape and measuring 0.47–0.53 mm. in length with a breadth of 0.13–0.17 mm.

Cervical papillae are situated 0.45 mm. from the anterior extremity.

Female. The tail is pointed; the vulva is situated 0.45–0.75 mm., the anus 0.15–0.2 mm. from the posterior extremity, the different measurements being due to the various degrees of contraction of the caudal region.

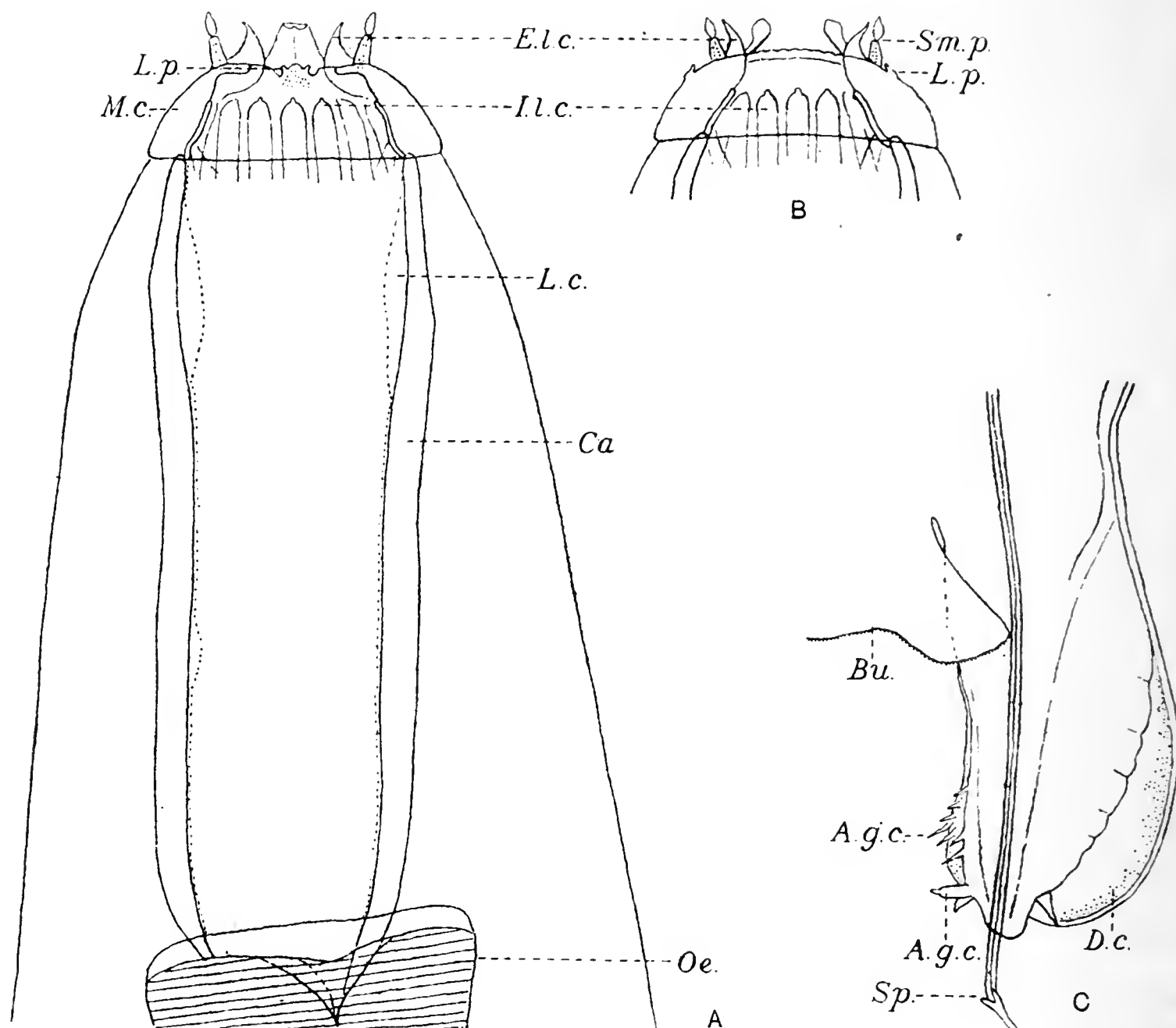


Fig. 1. *Cylandropharynx brevicauda* Leiper. A. Anterior extremity in lateral view. B. Anterior extremity, dorsal view $\times 215$. C. Genital cone and adjacent structures in the male, \times about 125.

Male. As described by Leiper, the males of this species are characterised by the large genital cone, the latter is almost cylindrical (up to 0.4 mm. in length with a breadth of 0.17 mm.) and completely surrounded by the dermal collar which, however, shows its greatest development on the ventral surface (Text-fig. 1, c).

The appendages of the genital cone are quite peculiar, a pair of finger-shaped appendages with rounded ends occur just behind the genital opening, whilst an irregular number of delicate pointed processes are scattered over the dorsal surface of the cone (Text-fig. 1, c).

As in *Triodontophorus* the bursa has a denticulated margin, the dorsal lobe measures about 0.2 mm. in length, the lateral lobes are narrower than those of *C. longicauda* and do not completely embrace the genital cone. The external branch of the posterior ray is deeply bifurcated (Text-fig. 2, c).

The spicules are long (about 1.1 mm.) and rather stout, they have hook-like terminations recalling those of *Triodontophorus*.

***Cylindropharynx longicauda* Leiper, 1911.**

Four specimens of this species occur in the Nairobi material, two males and two females, the former measuring 4.7 and 5.8 mm. in length, the latter 6.2 and 7 mm. respectively. The greatest breadth of the body is about 0.45 mm.

The head has a breadth of 0.14–0.15 mm. The mouth-collar and the leaf-crowns resemble those of the type species.

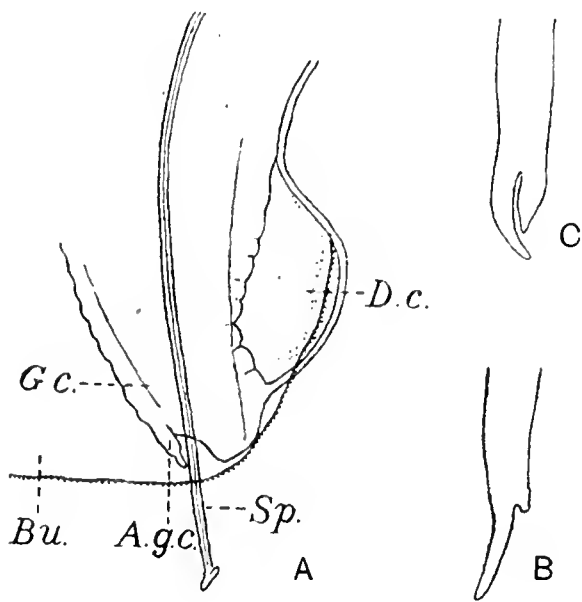


Fig. 2. *Cylindropharynx longicauda* Leiper. A. Genital cone of the male. B. External branch of the dorsal ray. C. *C. brevicauda*, external branch of the dorsal ray, \times about 125.

The mouth-capsule is smaller and considerably shorter than that of *C. brevicauda*, measuring 0.18–0.2 mm. in length, with a breadth of 0.07–0.09 mm.

The oesophagus is almost as long as in the type species (0.42–0.45 mm.), but much more slender, the greatest breadth being 0.08–0.1 mm.

Female. In the specimens before me the vulva opens 1.1 and 1.2 mm., the anus 0.28 mm. from the posterior extremity. The tail is long and pointed.

Male. The bursa is broader than in *C. brevicauda*, measuring nearly 0.5 mm. in a lateral view. The lateral lobes enclose the genital cone which is shorter and more globular (Text-fig. 2, A) than in that species. Leiper describes the external branch of the posterior ray as undivided, in the Nairobi specimens a small branch is given off close to the termination of the ray in the same position as the much longer branch of *C. brevicauda* (Text-fig. 2, B).

The appendages of the genital cone consist of a single pair of rather stout, finger-shaped processes.

The spicules are shorter (0.78 mm.) and more slender than those of the type species and are provided with less powerful terminal hooks.

As pointed out by Leiper, the species closely resembles *C. brevicauda*, the chief points of difference being: the smaller mouth-capsule, the more slender oesophagus, the elongated caudal region of the female and the structure of the bursa and the genital cone in the male.

Genus CYLICOSTOMUM Railliet and Henry, 1902.

Cylicostomum zebrae sp. n.

SPECIFIC DIAGNOSIS. *Cylicostomum*. A fairly large, robust species represented in the collection by three specimens, only two of which are adults, one female and one male, measuring 9.8 and 8.5 mm. in length respectively. The maximum thickness in both sexes is about 0.8 mm. Both worms have con-

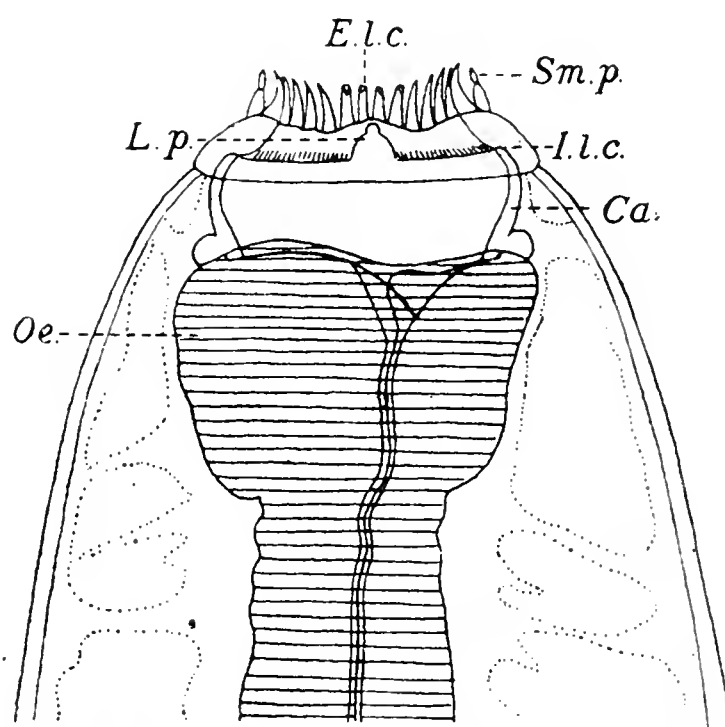


Fig. 3. *Cylicostomum zebrae* sp. n. Anterior extremity in lateral view, \times about 125.

siderably shrunk during fixation as is shown by the ratio of length to breadth, during life they must have been considerably more elongated than the figures show. The head is broad (0.27–0.3 mm.), not separated from the body by a neck constriction. The mouth collar is relatively low and depressed at the margins except in the neighbourhood of the lateral papillae. The latter are large and very prominent, forming a conspicuous feature in a dorsal or ventral view of the head (Text-fig. 4).

The submedian head papillae are long, with leaf-shaped terminal appendages.

The mouth is oval in shape, the longer axis being directed dorso-ventrally. The external leaf-crown consists of about 32 pointed leaves, the elements of the internal leaf-crown are very inconspicuous and very small, appearing as a ring of fine striae immediately in front of the mouth-capsule (Text-fig. 3).

The mouth capsule is short measuring 0.07 mm. in length, with a breadth of 0.2 mm., its walls are thin except in the posterior region where they are

greatly thickened to form a "hoop-like" ring similar to that described by Looss (1901) in *C. elongatum* and *C. auriculatum*. There is no dorsal gutter.

The oesophageal funnel is very large and enclosed in the broad anterior end of the oesophagus, the latter, 0.75–0.85 mm. in length, is 0.2–0.25 mm. wide in the region of the funnel, narrowing to 0.12 mm. at the level of the nerve-ring and broadening out again to 0.23–0.25 mm. in the terminal region (Text-fig. 4).

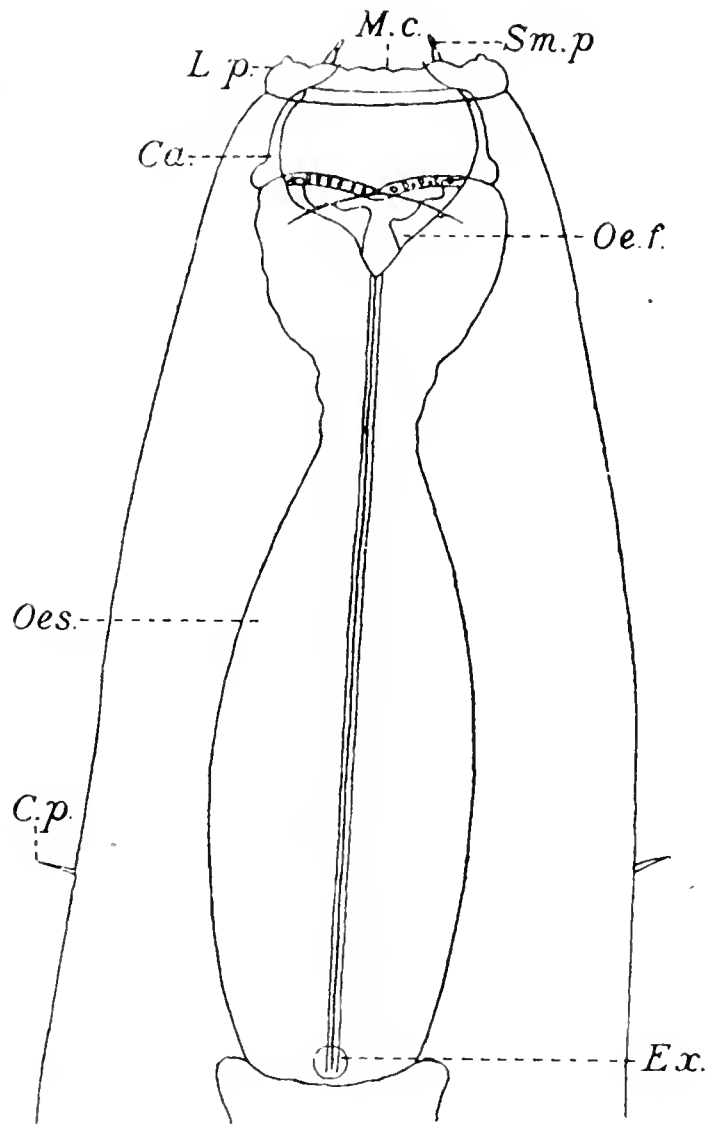


Fig. 4. *Cyclostomum zebrae* sp. n. Ventral view of the anterior extremity, \times about 75.

The chitinous lining of the oesophageal funnel is highly developed forming tooth-like ridges, but owing to the thickness of the oesophagus in this region their exact arrangement could not be ascertained, they are, however, indicated in Text-fig. 4.

The excretory pore opens at the level of the junction of oesophagus and intestine. The cervical papillae are a little further forward, 0.8 mm. from the anterior extremity of the body.

Female. The vulva is 0.25 mm. from the posterior extremity, at this level the body has a thickness of 0.23 mm. The anus is 0.1 mm. behind the vulva. Posterior to the anus the depth of the body decreases suddenly, forming a flat triangular tail. The vagina has a length of about 0.5 mm.

Male. The bursa (Text-fig. 5) is very similar to that of *C. insigne* Boulenger; the dorsal lobe is large and broad. The dermal collar is well developed

and surrounds the genital cone. The appendages of the genital cone are in the form of broad oval plates, fused together in the middle line.

This form evidently belongs to the group of species which includes *C. auriculatum* Looss, *C. elongatum* Looss and *C. insigne* Boulenger, agreeing with these in the general formation of the head and in the structure of the male bursa and its appendages. Apart from minor characters it differs from these species in the shape of the oesophagus with its enlarged anterior extremity and the greater development of the oesophageal funnel.

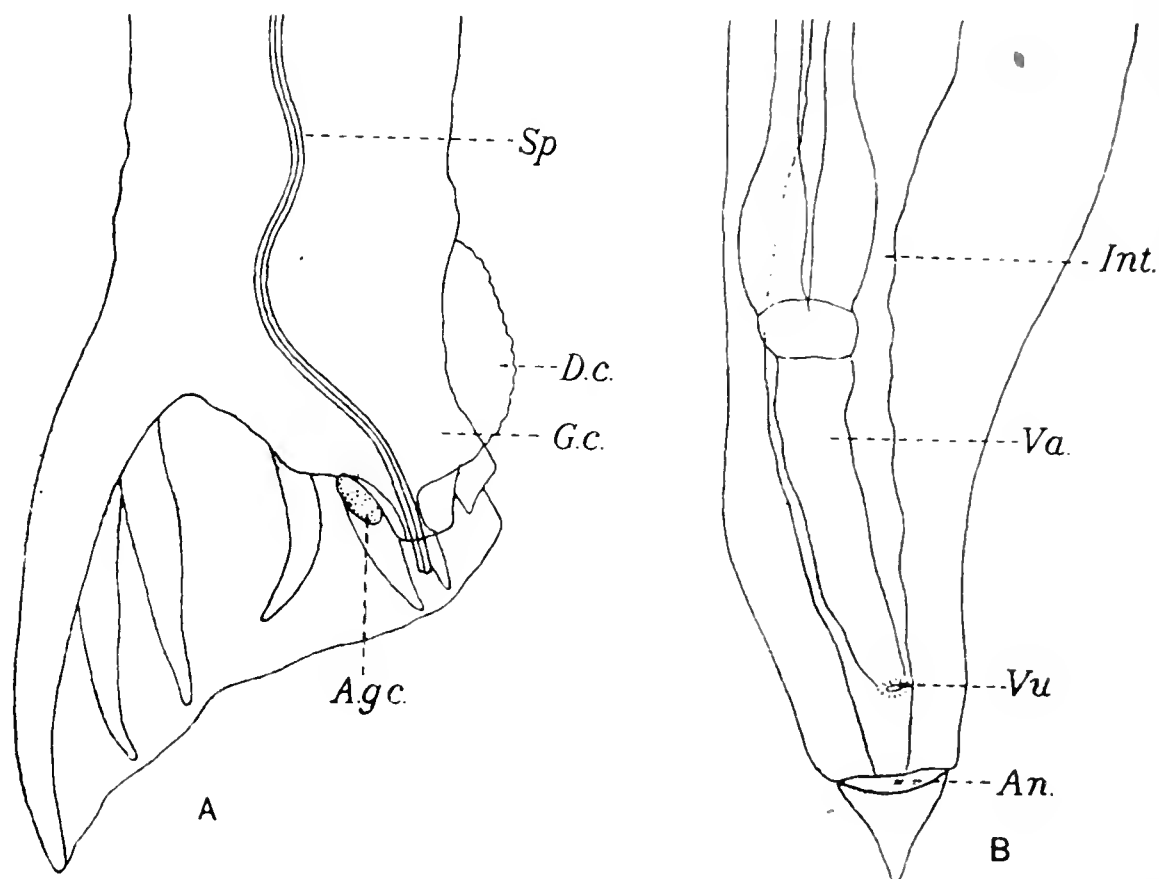


Fig. 5. *Cylicostomum zebrae* sp. n. A. Bursa of male from right side. B. Posterior extremity of female, ventral view, \times about 75.

***Cylicostomum Montgomeryi* sp. n.**

SPECIFIC DIAGNOSIS. *Cylicostomum*. A small species, females 4.5–6.5 mm., males 4.3–6 mm. in length. The greatest thickness of the body is 0.28 mm., decreasing to 0.2 mm. at the level of the commencement of the intestine.

The head is 0.08–0.09 mm. broad, not separated from the body by a constriction to form a neck.

The mouth is oval, the dorso-ventral axis being a little shorter than the lateral. The mouth collar is thick and depressed at the margins (Text-fig. 6).

The lateral papillae are prominent, the submedian slender with leaf-shaped appendages.

The anterior leaf-crown consists of about 18 slender, pointed leaves, the posterior leaf-crown of twice that number of rather similar but shorter elements (Text-fig. 6).

The mouth-capsule is characterised by its peculiar bilateral symmetry, its dorsal and ventral walls being considerably higher (0.032 mm.) than the lateral walls (0.022 mm.); this absence of radial symmetry makes an optical

section of the head in a lateral view present a totally different appearance to that viewed dorso-ventrally (Text-fig. 6, A and B).

There is no dorsal gutter. The oesophageal funnel is poorly developed.

The oesophagus is slender with an average length of 0.33 mm. Cervical papillae are present at the same level as the excretory pore, 0.24 mm. from the anterior extremity.

Female. The vulva is 0.13 mm., the anus 0.06 mm. from the posterior extremity of the body. The tail region behind the anus is narrow and ends in a fine point.

Male. The bursa is 0.25 mm. broad when viewed from the dorsal or ventral surface, the dorsal lobe is of medium length (0.12 mm.). The dermal collar is poorly developed and almost flat on the ventral surface of the genital cone. The latter is short, its appendages are similar to those of *C. tetracanthum* (Mehlis), *i.e.* ovoid in shape with a short blunt point on the posterior margin.

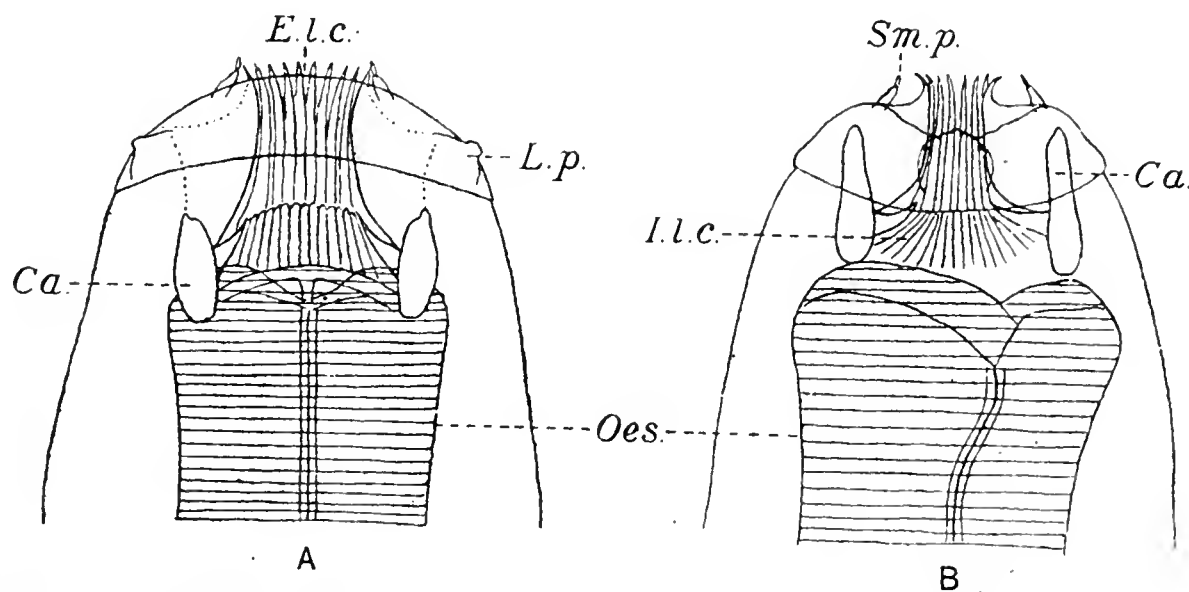


Fig. 6. *Cylicostomum Montgomeryi* sp. n. Anterior extremity. A. Dorsal view. B. Lateral view, \times about 350.

In the structure of its mouth capsule this form differs from all other known species of *Cylicostomum* with the exception of *C. falciferum* (Cobbold, 1882), a parasite of the Elephant now placed in a separate genus *Murshidia* Lane, 1914. In the latter species, however, the bilateral symmetry extends to the elements of the leaf-crown; this is not the case in *C. Montgomeryi*, where these structures are radially arranged.

Genus CRATEROSTOMUM gen. n.

Type species: *C. tenuicauda* sp. n. Closely allied to *Triodontophorus* Looss, differing from this genus by the absence of teeth projecting into the mouth-capsule.

Craterostomum tenuicauda sp. n.

Small, rather robust worms; the type specimens consist of three females only, 4–5.5 mm. in length. The cuticle of the body is transversely ringed. Body with a maximum breadth of nearly 0.4 mm., reduced to 0.28–0.32 mm. at the level of the termination of the oesophagus.

The head is 0.17 mm. broad, not separated from the body by a constriction.

The mouth is very small and circular, the mouth-collar narrow and depressed at the margins (Text-fig. 7, A).

The lateral papillae are not prominent, the submedian papillae small with leaf-shaped terminations.

The external leaf-crown consists of nine comparatively large almost triangular leaves arising from the inside of the mouth-collar as in *Triodontophorus* spp. The elements of the internal leaf-crown are also similar to those found in that genus, being septa-like projections, numbering 18 only.

The mouth-capsule and dorsal gutter (Text-fig. 7, A) are also similar in structure to those of *Triodontophorus*, the former is a little broader than long, measuring 0.07×0.05 mm.

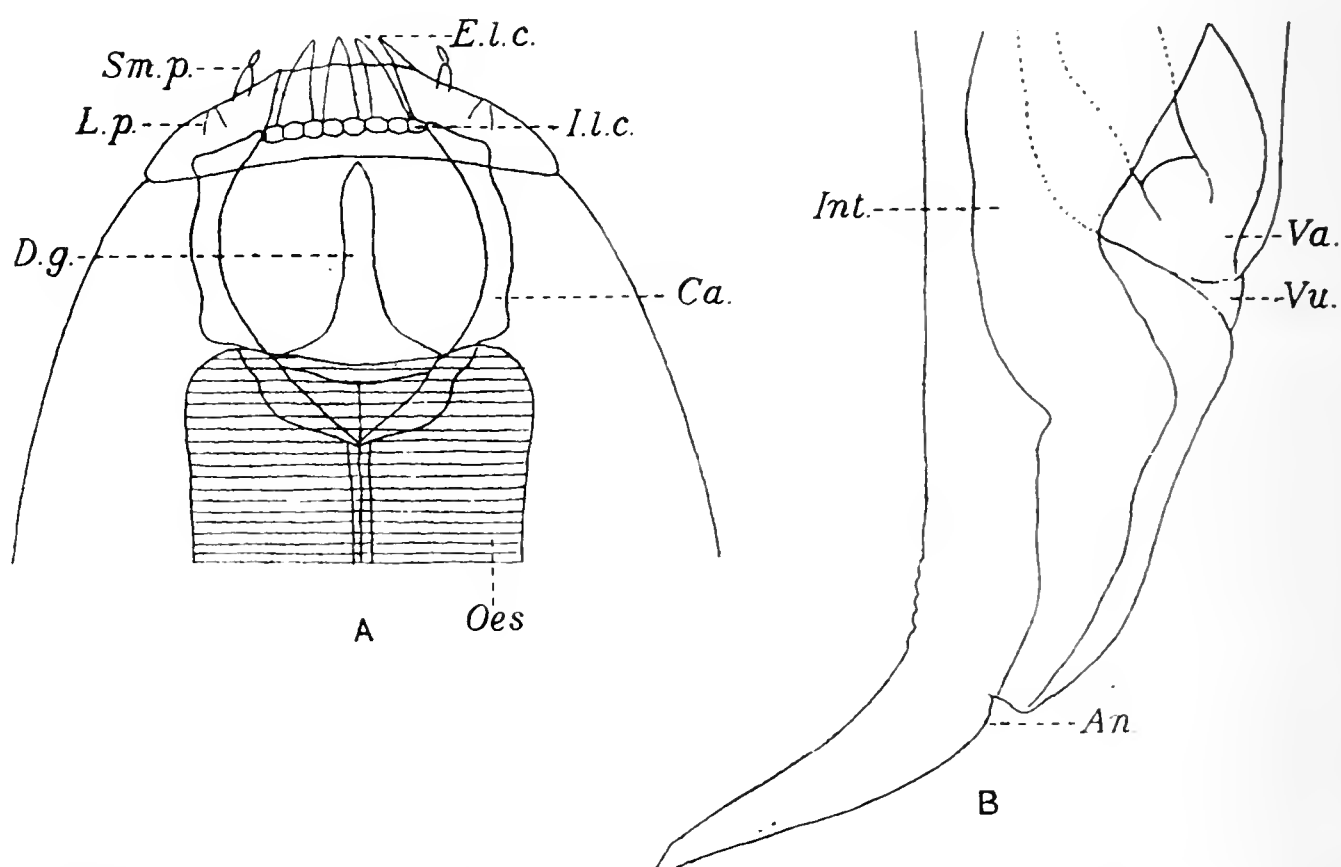


Fig. 7. *Craterostomum tenuicauda* gen. et sp. nn. A. Anterior extremity, ventral view, \times about 350. B. Posterior extremity of female, lateral view, \times about 125.

The oesophageal funnel is poorly developed and there are no teeth projecting from it into the mouth-capsule. The funnel is similar to that found in many species of *Cylicostomum*.

The oesophagus is narrow and has a length of 0.37–0.4 mm. Excretory pore and cervical papillae are at the same level, about 0.25 mm. from the anterior extremity.

The female genitalia are similar to those of some species of *Triodontophorus*. The vagina is very short (Text-fig. 7, B). The vulva opens 0.53–0.57 mm. from the posterior extremity; at this level the body has a thickness of 0.2 mm., this is reduced to 0.07–0.08 mm. in the anal region, 0.25–0.27 mm. from the end of the body. The tail region behind the anus is much attenuated (Text-fig. 7, B) and terminates in a point.

Although vagina and uteri are well developed in all the specimens, these as yet contain no mature ova.

In the majority of its characters this species very closely resembles certain species of *Triodontophorus*, and is only excluded from that genus on account of the absence of teeth in the mouth-capsule. The mouth is relatively smaller and the number of elements of the leaf-crowns considerably less than in any known species of *Triodontophorus*.

On account of the small size of the type-specimens and the absence of ripe ova, these worms might be considered as young stages of some species of *Triodontophorus*. This seems to me highly improbable, the specimens before me have evidently undergone the final moult and are obviously almost fully developed even if they have not reached their complete size. Species of *Triodontophorus* at this stage have the capsule-teeth fully developed, I have seen these structures in specimens smaller than those described above.

The absence of these teeth therefore makes it impossible to include the worms in the genus *Triodontophorus* in spite of their close affinity; I have therefore been obliged to regard them as the types of a new genus.

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EXPLANATION OF LETTERING IN TEXT-FIGURES 1-7.

<i>A.g.c.</i>	Appendage of the genital cone.	<i>I.l.c.</i>	Internal leaf-crown.
<i>An.</i>	Anus.	<i>Int.</i>	Intestine.
<i>Bu.</i>	Margin of the bursa.	<i>L.p.</i>	Lateral papilla.
<i>Ca.</i>	Wall of mouth-capsule.	<i>M.c.</i>	Mouth collar.
<i>C.p.</i>	Cervical papillae.	<i>Oes.</i>	Oesophagus.
<i>D.c.</i>	Dermal collar of genital cone.	<i>Oe.f.</i>	Oesophageal funnel.
<i>D.g.</i>	Dorsal gutter.	<i>Sm.p.</i>	Submedian papilla.
<i>E.l.c.</i>	External leaf-crown.	<i>Sp.</i>	Spicule.
<i>Ex.</i>	Excretory pore.	<i>Va.</i>	Vagina.
<i>G.c.</i>	Genital cone.	<i>Vu.</i>	Vulva.

A PARASITIC SPIRAL ORGANISM IN THE STOMACH OF THE CAT.

BY R. K. S. LIM.

(From the Department of Physiology, University of Edinburgh.)

(With Plate VII).

WHILE examining sections of the apparently normal cat's stomach, clusters of organisms were found within the lumina of numerous ducts and glands—especially of the pyloric region. Further examination, employing various methods of staining, revealed the spiral nature of the organisms. The presence of large numbers suggested that they were actively growing and in the absence of obvious gastric disturbance in the cats in which they were found, it was concluded that they were non-pathogenic parasites.

The author has been unable to find any reference in bacteriological literature to such an organism in the cat, although Noguchi (1915-16) refers to the finding by Bell and Ruquet of a similar form of organism in the stomach of the dog. Spiral organisms have also been described by Lucet (1910) in a case of gastro-enteritis in the dog.

DISTRIBUTION.

Eight animals were affected: they had all been in the laboratory for some months. Cats which were killed immediately on admission or which had been isolated, were unaffected. Rabbits which had been kept in adjacent cages were not infected.

The stomach was the only organ in which the organisms (hereinafter referred to as "spirochaetes") were found, except a few in the duodenum, close to the pyloric sphincter. Preparations from the liver, spleen and bone marrow were negative. Within the stomach, the spirochaetes were found throughout the whole fundus, including the cardia, and in the pyloric antrum and canal. They were most numerous in the latter situation. It should be noted that in the cat, oxyntic cells are only absent within a narrow area, half to three-quarters of an inch, proximal to the pyloric sphincter, and that therefore the fundus and the major portion of the antrum are histologically, and probably functionally, similar. The term "fundus" in the following description is applied to the whole area of the stomach bearing oxyntic cells, and "pylorus" to the narrow area devoid of them.

In the fundus region, groups of spirochaetes may usually be seen within the lumen of the tubules in the middle zone of the mucosa, where the oxyntic cells occur most abundantly and where they frequently abut directly on the lumen. In sections stained with alcoholic eosin and methylene blue (*vide* Lim, 1919) and in those stained with polychrome methylene blue alone, spirochaetes can be shown within the oxyntic cells¹, lying either in large clear spaces, enclosed by a membrane or amongst the granules in the interior of the cells (see Plate VII, figs. 2 and 3). Deeper down in the mucosa, where there are few oxyntic cells, only isolated organisms are present. They are not seen in the interior of the central or peptic cells.

In the pylorus proper, dense masses of spirochaetes occur at all levels of the mucosa, but only within the lumina of the ducts and secretory tubules (Plate VII, fig. 1), which are extremely wide in this region. No spirochaetes are visible outside the glands, either in the mucous, submucous or muscular layers.

The presence of organisms does not alter the histological appearance of the stomach further than has been described above.

MORPHOLOGY.

Measurements. The spirochaetes have been found to average in length from 4 to 8 μ in preparations taken direct from the fresh stomach and examined immediately. In stained films, somewhat longer forms are sometimes met with. The breadth varies from 0.75 to 1 μ and the thickness of the spiral, which is cylindrical, from 0.25 to 0.5 μ . Some of the smaller spirochaetes are extremely slender and have a spiral thickness of less than 0.2 μ . The spirals are regular, and closely set together, there being usually about 7 to 8 spirals in each spirochaete of from 5 to 6 μ . Occasionally as many as 14 spirals may be present, especially in the large forms. The spirochaetes are generally straight, but may exhibit one or more waves or curves (see Figs. 1, 3 and 5). Both extremities are tapered. When compared with the organism found in the dog² (Pl. VII, fig. 4), the cat spirochaete appears to be nearly of the same size (perhaps a little shorter), but differs in having more numerous and more regular spirals.

Reaction to stains. The spirochaetes stain slightly with Gram, *i.e.* they are not perfectly alcohol-fast. They are readily stained by most aniline dyes, especially when any of the Romanowsky combinations are used. With Giemsa, they appear bluish, with alcoholic eosin and methylene blue, blue, and with polychrome methylene blue, violet or purple. For clearness of staining, polychrome methylene blue is undoubtedly the best, both for smears and sections; it should be applied for 2 to 3 minutes. The Levaditi method has given poor results in the author's hands. Stained specimens of

¹ I have received an unpublished diagram from Dr Murray, drawn in 1907, showing spirochaetes within the oxyntic cells of the dog's stomach.

² I am indebted to Prof. Ashworth for a smear preparation (and a drawing) made by Dr J. Murray in 1907, from which the above comparison was made and a photograph taken.

the spirochaetes often exhibit granules and vacuoles. The granules stain darkly with the basic dye and are undoubtedly chromidia; they are inconstant in number (see Pl. VII, fig. 5). Vacuoles are not nearly so common, and may be artefacts resulting from overheating. Very broad forms of the spirochaete are probably also due to this cause.

Behaviour of living spirochaetes. If a little of the gastric mucosa is scraped off and transferred to a slide, the spirochaetes may be easily studied without the use of the dark ground illumination. They are highly refractile and quickly catch the eye, by the distinctness and regularity of their spirals. One or two more highly refractile spots may be seen at one or both ends of some of the organisms, but, otherwise, they appear homogeneous and structureless. When not in progression, the spirochaetes are seen in various attitudes, either straight, curved like a semicircle, or more completely, like a loop, and show one or other of the following movements which may be very active:

(1) An intermittent corkscrew-like motion of their spirals, sometimes in one direction, and sometimes in the reverse direction. Occasionally, one end appears to turn more slowly than the other. (2) Large spiral movements, superadded to the smaller movements. (3) A circular sweeping movement of one extremity. (4) Lateral oscillation of the whole organism. (5) A rotary disturbance of the fluid medium at one end of the organism, although the visible extremity of the spirochaete is not in motion. This seems to suggest the presence of a fine terminal filament (flagellum).

The behaviour of some spirochaetes in the proximity of cells is interesting. They ram the cell and spiral or corkscrew furiously, occasionally giving large circular sweeps with their free ends. Suddenly they disengage themselves, turn a "somersault" and repeat the performance with the former free end. No spirochaetes, however, have been actually observed to penetrate a cell by this means. The method of progression is difficult to analyse, but it appears to consist of a combination of corkscrew and lateral movements.

CULTIVATION.

All attempts at cultivation have hitherto failed. Deep agar, cooked-meat, and gastric digests, with or without pepsin, HCl or serum, have been used with no success. Partial anaerobiosis was maintained in all cases, except when agar was employed, by means of a supernatant layer of liquid paraffin. The only result obtained, worthy of note, was that the spirochaetes may survive at least four days in an acid medium (0.02 per cent. HCl).

CONCLUSIONS.

From the incomplete data accumulated, it is not possible to classify the organism just described, but sufficient is known to regard it as a new species of the Spirochaetoideae. Morphologically, it resembles both the genus *Spiro-nema* and the genus *Treponema* (as defined by Noguchi, 1918). It reacts to

Giemsa in the same way as a *Spiroplasma* (stains bluish), but on the other hand, it is about the size of a *Treponema* and, like it, has regular spirals. Its exact position in the group must be left undetermined until more is known regarding its cultural reactions.

Of the mode of infection, nothing definite can be stated. The organism was not found below the pyloric sphincter and is certainly not passed in the faeces in a free living condition. Fleas were often found in the gastric content, but when examined microscopically (living specimens were also procured direct from the body surface), there was nothing to indicate that they were the carriers. This part of the investigation was however not sufficiently pursued to negative the "carrier" possibility. The food (fish, bread and milk) does not appear to be responsible for the infection, since animals which were recently admitted to the laboratory were fed on the same diet without being infected. Taking into consideration the length of time the infected animals had been kept in the laboratory, it seems most likely that the infection was introduced by a single animal, many months ago, and that this animal infected the others. The mode of spread from one animal to another is still to be explained.

I have to thank Professors Ashworth and Ritchie for kindly confirming my observations and for helpful suggestions. I am indebted to Professor Ritchie for the supply of some of the culture media.

The expenses of the research were defrayed by a grant from the Earl of Moray Fund of the University of Edinburgh.

SUMMARY.

1. A parasitic spiral organism averaging 4 to 8 μ long, with regular, closely set spirals about 0.75 μ broad, has been found in the stomach in eight cats, none of which showed any obvious signs of gastric disturbance. The organisms occurred in the lumina of ducts and glands throughout the stomach, and also within the oxyntic cells. They were not seen in any part of the intestines except the very beginning of the duodenum, or in any other organ.

2. They are extremely active, and are readily stained by aniline dyes.

3. The mode of passage from one animal to another is not known, but food and faeces may be eliminated as possible sources of infection.

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EXPLANATION OF PLATE VII.

- Fig. 1. Cat III. $\times 1000$. Polychrome methylene blue. Photograph. Section of pylorus, showing spirochaetes in the lumen of a duct.
- Fig. 2. Cat I. $\times 1000$. Drawing of selected oxyntic cells. (a) Oxyntic cell showing spirochaetes lying among the granules; (b) spirochaetes lying within a dilated canaliculus; (c) spirochaetes lying partly in the canaliculus and partly in the lumen of the gland.
- Fig. 3. Cat IV. $\times 1000$. Smear preparation. Alc. eosin and methylene blue. Photograph. This shows the variations in size.
- Fig. 4. Smear preparation from the Dog's stomach. $\times 1000$. Leishman. Photograph. Compare with preceding figure. Note that the spirochaetes here show larger spirals.
- Fig. 5. Cat VII. $\times 2000$. Smear preparation. Alc. eosin and methylene blue. Photograph. Spirochaetes showing chromidial granules.



Fig. 1

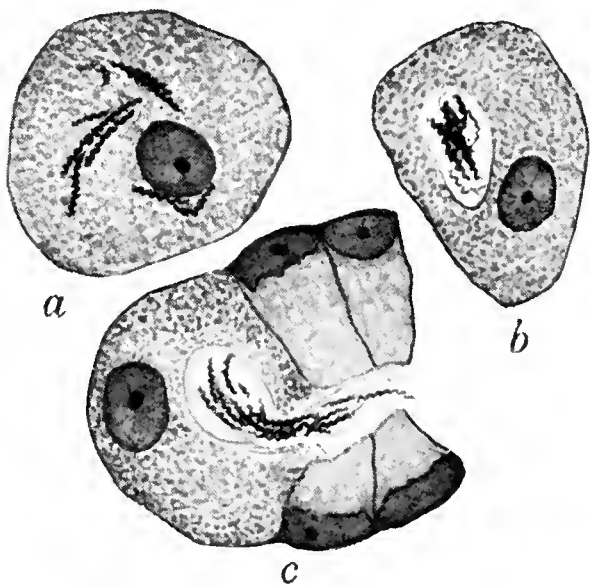


Fig. 2

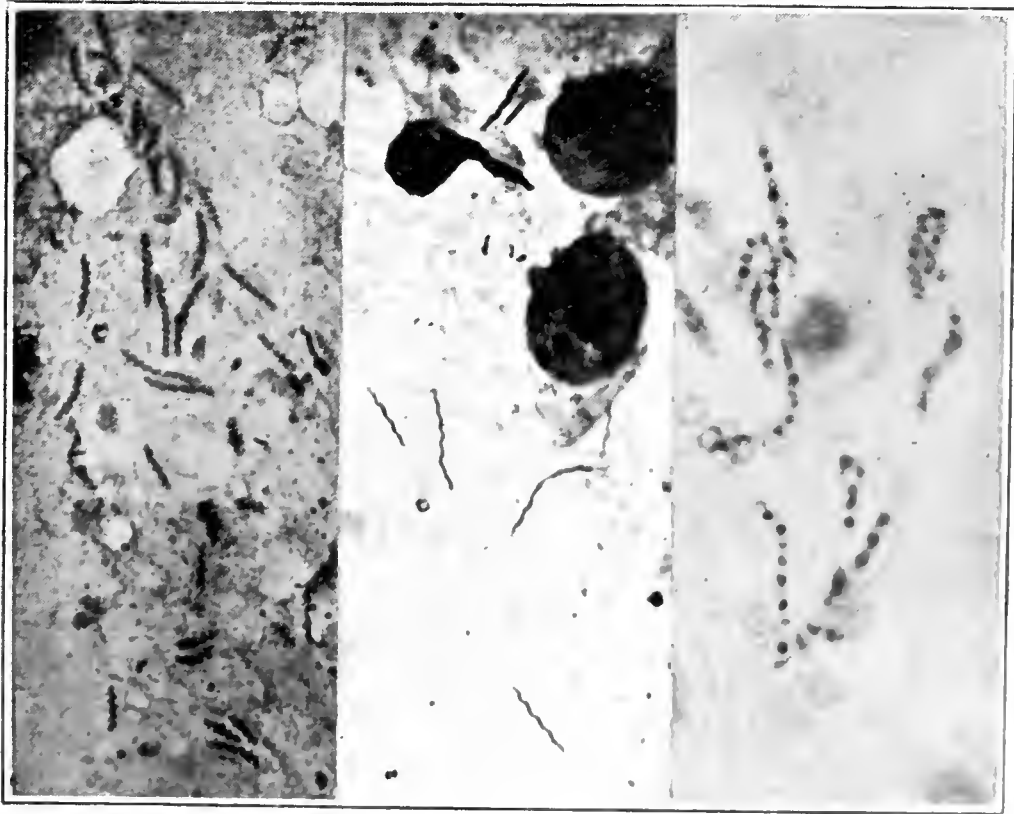


Fig. 3

Fig. 4

Fig. 5

ON A FILARIA, *LOA PAPIONIS* N. SP., PARASITIC IN
*PAPIO CYNOCEPHALUS*¹.

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(With Plates VIII and IX.)

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INTRODUCTION.

BEFORE the war, the yellow baboon (*Papio cynocephalus*) was imported in considerable numbers by Dr L. C. Quéry of Paris, who organized the capture of these animals by native hunters in the interior of French Guinea and their shipment from the port of Conakry. On arrival in Paris, they were utilized by him for the production of his antisyphilitic serum. Increasing doses of a filtered broth culture of the syphilitic virus were injected subcutaneously, the baboons being killed by bleeding from the carotid artery about a month later. Having noticed the occasional presence of round worms during the operation

¹ The old name of this species, *Cynocephalus babuin*, has been condemned for reasons of priority.

of exposing the carotid artery, Dr Quéry mentioned his discovery to the late Prof. R. Blanchard, who very kindly suggested my investigating the matter. The larval and adult forms of a filaria belonging to the genus *Loa* were identified in a certain proportion of the animals examined, and the problem as to their identity or otherwise with the human parasite *Loa loa* investigated. Certain experimental researches were also initiated, but in August 1914, my work was interrupted by the war and is therefore incomplete in certain respects; I hope, however, to make good these deficiencies later. The investigations detailed in this paper were made, partly in the Laboratory of Parasitology, partly in Dr Quéry's private laboratory, in Paris.

Acknowledgments. I have to thank Dr L. C. Quéry and his staff for much kind help on all occasions; also Dr M. Langeron, whose suggestions with regard to technique were often invaluable. I am indebted besides to Prof. R. T. Leiper for several suggestions. Plate IX, fig. 3 is from a water colour by Dr E. Miller; the remainder were drawn and coloured by Madame Blanchard.

I. Description of the adult form of *Loa papionis*.

The presence of cuticular bosses in both sexes is the most obvious characteristic of the genus *Loa*. The single species hitherto recorded is *Loa loa* Guyot 1778¹. The more important differences between this parasite and the one I am about to describe are summarized in Table I. I am inclined to think these differences justify the creation of a second species, and have accordingly named the worm *Loa papionis* after the baboon in which it was found.

Technique. The living worms were fixed in 70 per cent. alcohol at 60° C.; they were subsequently transferred to cold 70 per cent. alcohol where they were left until wanted. Before clearing, they were placed in 0.9 per cent. saline for some minutes to enable them to recover from the slight shrinkage caused by the alcohol: if this precaution be observed, the measurements made before and after fixation are identical.

The *neck* or *shoulder* described or figured by Manson (1912), Penel (1904) and Looss (1904) does not exist—at any rate in this species—provided the living worm be fixed in hot alcohol. However, this artefact is frequently visible after fixation in cold alcohol, and is no doubt produced by the contraction of the muscle cells inserted at the level of the base of the cephalic cone. In living worms an indentation appears at about this level—first on one side, then on the other—during movements of the head; it is never visible during rest.

External measurements. 12 ♂♂ and 12 ♀♀ were taken from the same baboon and measured before fixation. The ♂♂ measured 27 to 33 mm. in length, 0.4 to 0.5 mm. in breadth; the ♀♀ measured 63 to 83 mm. in length, 0.55 to 0.7 mm. in breadth. The maximum diameter is usually reached in both sexes

¹ Skrjabin (1917) describes a parasite to which he gives the name *Loa extraocularis*. However, the cuticular bosses and other characteristic features of the genus are absent from his description, which more nearly corresponds to the one usually given for *Filaria* (?) *conjunctivae* Addario.

at a point between 3 and 4 mm. behind the anterior extremity; this is maintained for a short though variable distance, after which the diameter gradually diminishes to the rounded posterior extremity. The breadth at the level of the anus is from 0.09 to 0.13 mm. in the ♂, 0.17 to 0.21 mm. in the ♀.

The figures recorded by Penel (1904), who found 34 *L. loa* at the autopsy of a negro, are of interest in this connection, and the differences between his observations and my own are brought out in Table I. In *L. papionis*, both sexes are considerably thicker and the females considerably longer than in *L. loa*; the smallest female measured by me was as long as the largest of Penel's specimens, while its maximum diameter was greater. On comparing worms of average size with one of Penel's—a female which forms part of the collection belonging to the Laboratory of Parasitology, Paris—these differences appeared very obvious.

Table I.
Summary of chief differences between Loa loa and L. papionis.
(For differences in the spicules, see text, p. 119.)

I. THE ADULTS.				
External measurements in mm.				
	Length	♂	Length	♀
		Maximum breadth		Maximum breadth
<i>L. loa</i> (Penel's figures, 1904)	25-34	0.273-0.43	45-63	0.38-0.49
<i>L. papionis</i>	27-33	0.4 -0.5	63-83	0.55-0.7

Maximum thickness of cuticle in microns.		
	♂	♀
<i>L. loa</i> Looss (1904)	9-12	9-12
<i>L. papionis</i>	10-12	16-20

Breadth of lateral bands in microns.			
	Breadth of lateral band at base of cephalic cone	Breadth a short distance behind base	Breadth at termination of oesophagus
<i>L. loa</i> (Looss's figures, 1904)	♂ 9	—	63
	♀ 9	—	90
<i>L. papionis</i>	♂ 20-35	10-15	50-60
	♀ 40-60	20-34	80-100

II. THE LARVAE.				
<i>Microloa loa</i>			<i>Microloa papionis</i>	
Sheath			Present	
Measurements in microns ...			Usually absent	
(Fresh material)			210-280 × 7-7.5 Low (1911)	
Shape ...			—	
Subcuticular cells ...			Numerous ...	
Central viscus ...			Seldom seen ...	
Periodicity ...			Diurnal ...	
			Posterior extremity rather more pointed	
			Scanty	
			Frequently present	
			No diurnal periodicity	

The cuticle and subcuticular layer. When fixed and cleared as above described, the cuticle is seen to be composed of two layers, of which the internal is thicker than the external. In the male, the internal layer shows an exceedingly fine ringed striation for the posterior 2 to 3 mm. which is visible both in the living worm and after fixation in hot alcohol; a less distinct but otherwise similar striation is sometimes to be seen in the female. The coarse striae described by Ludwig (1895) result from muscular contraction, and can be seen to appear and disappear during the movements of the living worm. They may be preserved in places after fixation in cold alcohol, but are never visible after fixation in alcohol at 60° C.

The maximum thickness of the cuticle is distinctly greater in the female than in the male: however, for *L. loa*, Penel (1904) and Looss (1904) give an identical measurement for both sexes (Table I). The cuticle is much thinner at the extremities (3 to 6 μ in the ♂, 4 to 8 μ in the ♀); moreover it is thicker over the anterior extremity than over the sides of the cephalic cone (Plate IX, fig. 1, *Cut.*). In the case of *L. loa*, observers are not in agreement on these points: Looss (1904) represents the cuticle as being of equal thickness in both situations, while Blanchard's drawing (1889) which is reproduced by Fantham, Stephens and Theobald (1916), more nearly corresponds to what I saw.

The cuticular bosses are very similar in appearance and distribution to those described for *L. loa*. They commence in both sexes at a variable distance behind the anterior extremity (0.71 to 2.5 mm. in the ♂, 1.2 to 3.5 mm. in the ♀). They are rather scanty in the ♂, being altogether absent from the posterior 0.54 to 1.7 mm.: numerous in the ♀, they frequently show a tendency to be concentrated in small groups, and always reach the tip of the tail or thereabouts. In both sexes the bosses vary in size in different specimens, but are always larger in the ♀ (Table II). They almost invariably look clear and structureless but I have seen the appearance of an internal papilla.

Table II.

Measurements of the cuticular bosses compared in both sexes.

		Height of bosses in microns	Diameter at base in microns
♂	No. 1	3- 6	8-14
	No. 2	3-10	8-20
	No. 3	3- 8	8-20
♀	No. 1	8-16	14-24
	No. 2	4-10	12-22
	No. 3	6-20	14-22

The size of the clear, anterior area—the so-called *cephalic cone*—(Plate IX, fig. 1, *c.c.*) varies slightly in different specimens, but in both sexes the base of the cone measures from two and a half to three times the length of the vertical diameter.

The thin subcuticular layer has the appearance of a syncytium. Its nuclei may be seen from time to time in any situation, but are especially distinct in the anterior millimetre of each lateral band, where they form an irregular double row.

The four longitudinal bands are formed by thickenings of this layer. The two lateral ones are always visible in the anterior millimetre, but apart from this are seldom very distinct; I never succeeded in tracing them the whole length of the body as Looss was able to do, moreover their breadth does not correspond with Looss's figures (Table I). The median bands are sometimes visible in the anterior millimetre; they are much narrower than the lateral bands.

The muscular system. The *muscle cells* are well developed and often show the appearance of a longitudinal striation. Those measured, varied from 1 to 3 mm. in length, by 0.03 to 0.04 mm. in breadth.

The nervous system. The anterior edge of the *circumoesophageal nerve ring* (Plate IX, fig. 1, *N.R.*) is situated about 0.15 mm. behind the anterior extremity in the ♂, and about 0.2 mm. behind it in the ♀. Its breadth is 0.03 to 0.04 mm. in the ♂, 0.04 to 0.06 mm. in the ♀. Two rounded masses containing nerve cells—the *lateral ganglia* (Plate IX, fig. 1, *L.G.*)—are sometimes to be seen embedded in its substance. Three *nerve bundles* (Plate IX, fig. 1, *N.B.*), of which the anterior is the largest, arise posteriorly on either side; they cross the body cavity and disappear in the parietes. Looss (1904) describes the nerve ring as being 0.025 mm. broad. Neither he nor other observers mention the presence of nerve cells.

The anterior papillae. The two larger ones are 8 to 10 μ in diameter, lateral to latero-median in position, and situated from 20 to 30 μ in front of the base of the cephalic cone; they have the appearance of being subcuticular, and never project above the surface; a strand of nerve fibres can sometimes be seen passing to each.

The four *submedian papillae* were never distinguished with certainty.

The digestive system. The *mouth* is a simple funnel-shaped depression in the cuticle.

The oesophagus averages 0.9 mm. in length in the ♂, 1.2 mm. in the ♀: its anterior portion is somewhat bulbous, while the diameter at the level of the constriction between the oesophagus and the mid-gut is very constantly 0.04 mm. in the ♂, 0.06 mm. in the ♀. A tube of gland substance extends from the base of the cephalic cone to the end of the oesophagus—the so-called *oesophageal gland* (Plate IX, fig. 1, *O.G.*). Circumferentially it is attached to and lies between the muscle cells, the gland substance projecting into the body cavity. These projecting masses are better developed laterally than dorso-ventrally, and are especially prominent objects just behind the base of the cephalic cone. Two ducts open into the oesophagus at the posterior border of the nerve ring, but are seldom visible.

Looss describes a large dorsal and smaller subventral glands. Other

observers simply mention the presence of glandular substance without giving any details.

The mid-gut and rectum correspond to Looss's description.

The ano-genital opening is 0.084 to 0.086 mm. from the posterior extremity in the ♂, while in the ♀ the anus is rather more than double this distance away.

The excretory system. I only once succeeded in finding the *excretory pore* in the ♀, and never found it in the ♂; it was situated 0.68 mm. behind the anterior extremity on the ventral surface. What appeared to be an excretory canal was sometimes visible in the substance of the lateral band.

The genital system in the male. Although a cursory examination reveals the presence of five pairs only, seven pairs of pedunculated and symmetrically situated *genital papillae* exist. The first three pairs are preanal, the last three postanal, while all six have an oval outline and diminish in size from before, backwards (Plate IX, fig. 2, $P_1, 2$, etc.). The fourth pair are adanal in position and slender in shape. I never noticed them in undissected specimens; however, after removal of the spicules and hemisection of the posterior extremity they caught the eye at once in both cases in which this little operation was successful (Plate IX, fig. 3, P_4); in the undissected state they seem to be hidden by the spicules. The seventh pair are also readily overlooked owing to their subterminal position on the ventral surface of the sharply curved tail. If a number of living males be observed in normal saline, the tail is seen to be strongly curved in almost all of them; in fact it never seems to be completely uncoiled, although the extent of the curve may vary considerably during movement. Even after fixation in hot alcohol the curvature persists, and in several cases I was unable to see a trace of the seventh pair before dissecting the posterior extremity. Sometimes however, one or both of these papillae are quite conspicuous in the undissected state; in the first case the muscle cells were probably contracting more vigorously on the one side than on the other at the moment of fixation, the posterior extremity becoming slightly twisted in consequence (Plate IX, fig. 2, P_7); in the second case the posterior extremity was much less sharply curved than usual.

At the time, I was unable to reconcile these observations with the descriptions of *L. loa* available in the literature. Looss describes five pairs of papillae and mentions the presence of "terminal filaments" in both sexes; however, lack of material prevented him from deciding whether those of the male were distinct papillae or not. Penel (1904) records the presence of two little tubercles in this situation, as do Annet, Dutton and Elliot (1901). Penel also mentions the presence of small, adanal tubercles; but he gives no details, and does not say whether he regarded them as being constant or not. After reading Leiper's paper (1913), these difficulties largely disappear. Both Leiper and Lane regard the slender fourth pair as being bilaterally symmetrical and constant in *L. loa*; Leiper also seems to recognize the seventh pair as being pedunculated, and describes other small unpaired genital papillae in addition.

Taking everything into consideration, the number and arrangement of the paired genital papillae would seem to be identical in the two species.

The *spicules* (Plate IX, fig. 2, *Spic.*) differ from those of *L. loa* in being longer and broader, while the proximal ends of both are striated¹. The appearance of their free extremities differs widely from Looss's description, but agrees in some respects with that given by Leiper.

The larger of the two, which is also the more curved, varies from 0.18 to 0.246 mm. in length. Its proximal portion is very refringent and has a coarsely striated appearance both before and after removal from the body; the non-striated terminal portion tapers towards its free extremity, which is sharply pointed. The smaller spicule varies from 0.108 to 0.13 mm. in length. Its proximal portion is not especially refringent, but shows fine, transverse striae which are only visible after the spicule has been dissected out (they are indicated for a short distance by dark shading in Plate IX, fig. 2); the terminal portion is fairly substantial and ends rather bluntly, the tip somewhat resembling the end of a crochet needle. The basal portions of both spicules appear to have a fine lumen; however, their terminal portions are not canalised but grooved, the groove extending almost to the free extremity; in the case of the smaller spicule, the proximal end of the groove shows a small fusiform dilatation.

Both these organs are contained in sheaths which open at the ano-genital orifice; they may be seen protruding for a variable distance or may be completely retracted into the body; when projecting to any extent they tend to cross one another. Retractor muscles are inserted into their bases, the spicular sheaths being lost on their outer surfaces.

The *internal genitalia* consist of a tubular *testis* which terminates in the *ejaculatory duct*; this opens together with the *rectum* at the *anogenital orifice*.

The nearly spherical *spermatozoa* measure from 5–7 μ in diameter.

The *genital system in the female*. The position of the *vulva* is relatively constant, being never less than 2 or more than 3 mm. from the anterior extremity.

The length of the *vagina* is less constant. Ludwig gave it as 3 mm. for *L. loa*. All subsequent workers give it as 9 mm. and cast much doubt upon this observation of Ludwig's. After measuring several which varied between 9 and 10.5 mm. in length, I came across one which measured only 4.2 mm., so that personally I have little doubt as to the correctness of Ludwig's statement. The upper end of the vagina divides into an anterior and a posterior *genital tube*, each of which is subdivided by Looss into *uterus*, *receptaculum seminis*, *oviduct* and *ovary*. He gives a detailed account of these structures with which my own observations entirely agree; I will not therefore describe them.

¹ In *L. loa*, striae are only described for the larger spicule.

II. Description of the ova and larvae of *Loa papionis*¹.

Technique. I shall confine myself to pointing out a few slight modifications in technique which seemed to me to increase the efficiency of certain of the methods available in 1914. For further details, see Langeron (1916).

I. *The staining of dry blood-films.* When using Pappenheim's panoptic method I obtained the best results by staining for twenty minutes with a solution of Giemsa of half the ordinary strength.

II. *Wet fixation.* Looss (1914), in describing his method, says that the film is apt to become detached if slides are used and recommends coverslips; I have not found this to be the case and prefer slides, which are easier to manipulate.

III. *Treatment after wet fixation.* Although Heidenhain's iron haematoxylin method is usually employed after wet fixation, Giemsa, Azur II, and Carbol-methylgreenpyronin also give excellent results. Preliminary overstaining, followed by differentiation with 90 per cent. alcohol, enable every detail to be seen at one stage or other of the process; moreover preparations which keep, at any rate for some months, may be made by mounting in paraffin oil and cementing the edges of the coverslip.

IV. *Mensuration.* The measuring of living embryos or larvae is rather tedious, and it was found impossible to determine their dimensions with absolute accuracy owing to the waves of contraction which are constantly passing along their bodies. However the measuring of the dead material

¹ The difficulties in the way of an accurate naming of the forms circulating in the blood, are considerable. For example, in the nematode family we are considering, it is sometimes impossible to say whether the sheath present in blood-films represents the true egg-membrane or a moult; *i.e.* we are unable to say whether the organisms in question are eggs or not. Moreover the term "*embryo*" is often used rather loosely, descriptions of sheathed and sheathless embryos being commonly met with, both in the literature and in text-books. Again, Stephens (1916) asserts that in many parasitic nematodes the young must be called "*larvae*" even before they have left the egg-shell, for they present characters which are subsequently lost. Under these circumstances conventions are essential if confusion is to be avoided. In this paper, I shall use the terms "*ovum*" and "*embryo*" so long as the existence of the egg-membrane is certain. When the membrane is absent, or when it is doubtful whether the membrane present represents a moult or not, I shall use the term "*larva*." In other words:

(a) The use of the terms "*ovum*" and "*embryo*" is restricted to the forms present in the female genital tract.

(b) Sheathless forms from the genital tract, together with all forms present in the blood of the definitive host, are referred to as "*larvae*."

Yet another difficulty occurs when the adult form is unknown. This was originally met by the coining of the term "*microfilaria*" qualified by some non-committal name—*Microfilaria diurna* for example. At a later period, however, *Mf. diurna* was identified as being the offspring of *Filaria loa*, while a new genus was constituted consisting of a single species—*Loa loa*. In consequence of these discoveries and changes in nomenclature, *Microfilaria diurna* is now correctly referred to as *Microloa loa*.

before fixation cannot be recommended, for a certain amount of alteration has inevitably occurred, while the extent of this will vary with the time that has elapsed since death, with the nature and temperature of the surrounding medium and so on. These difficulties are best surmounted by fixing the living material in hot alcohol, the slides being transferred to 0·9 per cent. saline for purposes of mensuration. The chief advantages of this procedure are the following:

- (1) Simplicity of technique.
- (2) The shrinkage that occurs is uniform and relatively slight.
- (3) The taking of accurate measurements is greatly facilitated because most of the larvae are fixed in complete extension.
- (4) The possibility of their dimensions being altered by complicated staining processes is avoided.

A glance at Table III shows how greatly these measurements are affected by the conditions under which they are made. Since the figures obtained from the mensuration of dried material are obviously valueless, it would undoubtedly simplify matters if those obtained after fixation in 70 per cent. alcohol at 60–65° C. and examination in 0·9 per cent. saline were stated as often as possible in addition to the figures obtained from living material.

Table III.

Measurements of Microloa loa and Microloa papionis compared in microns.

	<i>Ml. loa</i>		<i>Ml. papionis</i>	
	Length	Breadth	Length	Breadth
Fresh material	210–280	7–7·5 Low (1911)	270–330	6·5–7
Smears fixed wet in hot alcohol ...	208–254 Stephens (1916)	—	270–330	About 5
Dry unfixed smears treated with azur II	—	—	245–310	—
Dry smears fixed with absolute alcohol	131–166·5 Stephens (1916)	—	178–265	—

Morphology of the larvae taken from the blood. In fresh preparations, the movements of the larvae are indistinguishable from those of *Ml. loa*. After dry fixation, they are never disposed in such graceful curves as the larvae of *Filaria bancrofti*; both in thin and thick smears their attitude more nearly resembles that of *Ml. loa*, but their tails are rather more pointed (Plate VIII, fig. 1). I seldom noticed the presence of a *sheath*, although it was occasionally very distinct both in fresh and in stained preparations; as in *Ml. loa*, it is comparatively short and keeps its form during the movements of the larva.

The *cuticle* is transversely striated, and its appearance closely corresponds to Fülleborn's (1913) diagram of the cuticle in *Ml. loa*. The nuclei of the *subcuticular cells* are few in number (Plate VIII, figs. 3, 4, *N.S.C.*); I never saw

more than six or seven in the same larva, whereas Fülleborn represents forty-one in his diagram.

The anterior 7 to 10 μ is free from nuclei. In living larvae, a rather refringent terminal or subterminal granule is often visible at the anterior extremity, while an oval rather clear area, resembling a vacuole, is frequently present immediately behind it. These structures are not individualised in stained smears; however, in dried blood-films stained by the panoptic method, a central red streak or series of dots is sometimes present in this clear cephalic area.

In unfixed films stained with Giemsa, two pairs of bright-red elongated structures are often visible—one pair at each extremity (Plate VIII, figs. 5, 6, C.B.). Although Fülleborn describes them in *Ml. loa* and recommends vital staining with azur II and eosin for their demonstration, I never succeeded in staining them by this method; I occasionally noticed them, however, in dry films after fixation in absolute alcohol and staining with Giemsa. The column of body nuclei is separated by a space from the cuticle: the first nuclei are frequently situated in the same transverse plane, while the last one reaches the tip of the tail or thereabouts.

The *nerve ring* and the *pore-chambers* with their *pores* show nothing distinctive; in fresh material examined in a 1 in 2000 solution of azur II in 0.9 per cent. saline, the stain was observed to enter by the pores as is the case in other species.

The appearance presented by the *excretory* and *genital cells* (Plate VIII, figs. 2, 3, 4, *Ex.c.*, *G.C.*) agrees quite closely with the descriptions given by Rodenwalt (1908, 1909) and Fülleborn (1913, 1914) for the corresponding structures in *Ml. loa*. The *subsidiary genital cells* are seldom visible. The average position of the more important anatomical landmarks is given in Table IV.

Table IV.

Position of the more important anatomical landmarks expressed in percentages of the body length.

Middle of nerve ring	...	20.3
Excretory pore	30.3
Nucleolus of excretory cell		34.3
Nucleolus of first genital cell		66.5
Anal pore	81.7

In dry blood-films stained by the panoptic method, a *tail spot* is usually present immediately behind the place where the last two nuclei occur abreast (Plate VIII, fig. 1, *T.S.*).

The *central viscus* (Plate VIII, figs. 1, 2, 3, C.V.) is present in a fair proportion of the larvae and is often visible in living unstained ones. It lies a short distance in front of the first genital cell and varies up to 45 μ in length. In dried

blood-films stained by the panoptic method, it appears as a reddish granular mass, but after wet fixation the granular appearance is less in evidence; in smears treated with iron haematoxylin it appears as a siderophile body, while it stains red with carbolmethylgreenpyronin. The number of nuclei is considerably reduced in the region of the central viscus and first genital cell (Plate VIII, fig. 4); in stained preparations the resulting light area varies from 50 to 60 μ in length.

Morphology of the ova and larvae taken from the female genital tract. The ripe ovum measures about 25 by 18 μ . The egg membrane is secreted immediately after the entrance of the spermatozoon (Plate VIII, fig. 7, *E.M.*). At the stage of commencing differentiation the egg measures 41 by 28 μ , intermediate measurements corresponding to the morula stage. The egg grows in size up to 55 by 37 μ when the embryo commences to uncoil; the terminal flexure of the tail represents the last stage of the uncoiling process, and larvae showing this peculiarity are sometimes present in the blood-stream. Uncoiled embryos are possibly a little narrower than larvae from the blood, but their length varies between the same extremes. The egg membrane persists for a time as the embryonal sheath, but a variable proportion of sheathless larvae are present in the vagina and lower ends of the uterine tubes. I never succeeded in demonstrating the central viscus in young embryos, while it was small or absent during the uncoiling process; at this stage the anal pore chamber is not always visible, while the subsidiary genital cells are not to be seen; the nerve ring is present, but small. Little can be made out in the coiled up stage; but the excretory pore chamber is sometimes visible. Apart from such differences, all of which result from examination at an earlier stage of development, the structure of the embryos from the genital tract was identical with that of the larvae found in the blood.

The chief differences between *Microloa papionis* and *Microloa loa* are summarized in Table I.

A CONSIDERATION OF SEVERAL IMPERFECTLY SOLVED MORPHOLOGICAL PROBLEMS.

The sheath. In preparations made from the lower end of the genital tract of adult females the sheath was usually, but by no means invariably present, while it was seldom seen in blood-films. At first sight this seems rather remarkable, for the sheath appears to have been regarded by all observers as invariably present in blood-films containing *Ml. loa*. Is it possible that the presence of a sheath in *Ml. loa* is not such a constant morphological feature as it is alleged to be? The following facts would seem to be of interest in this connection:

(1) According to Stephens (1916), the uteri of *L. loa* contain eggs in the most various stages of development, besides hatched-out larvae.

(2) Brumpt (1913), remarks that in stained preparations the sheath stains poorly and therefore often appears to be absent. However, other writers do not emphasize this point, and it is certain that, on occasions, the sheath of *Ml. loa* may be very prominent in stained preparations.

(3) Manson (1912), represents *Ml. loa* as having the extremity of the tail sharply bent, while Fülleborn denies this to be the case. I seldom noticed this peculiarity in larvae from the blood, but it was frequently present in embryos taken from the female genital tract. This flexion of the tail clearly represents the terminal stage of the uncoiling process, and the microfilariae figured by Manson must be assumed to have reached the blood at a rather earlier stage than is usually the case.

On the whole, it looks as though the sheath in *Ml. loa* represents a moult rather than a persistent egg membrane in a certain proportion of cases, while statements concerning its invariable presence must, temporarily at any rate, be regarded with a certain amount of scepticism.

The buccal apparatus. The structure of the buccal apparatus—if it really exists—is most obscure. Manson (1912) gives the following description for *Mf. bancrofti*. “When the movements of the living microfilaria have almost ceased, by careful focussing it can be seen that the head end is constantly being covered and uncovered by a six-lipped—or hooked—and very delicate prepuc; moreover one can sometimes see a short fang of extreme tenuity based apparently on a highly refractile granule, suddenly shot out from the uncovered extreme cephalic end, and as suddenly retracted.” Recent observers seem sceptical as to the existence of these structures. Looss (1914) frankly puts them down as optical phenomena. Fülleborn (1913) is doubtful about the existence of the prepuc and thinks the retractile appendage described by Manson was probably the upper edge of the sheath.

Personal observations. In fresh preparations I not infrequently noticed a terminal or subterminal spot or granule, together with an elongated, rather clear area behind it; this granule was often very refringent, while both it and the clear area were frequently visible in dead as well as in living larvae. In dried smears stained by the panoptic method, a central red streak or series of dots was sometimes present in this situation.

In the living larva I once observed an appearance of rhythmic movement a short distance behind the anterior extremity, but saw no denticulations. From time to time a filament appeared to be shot out and retracted for a distance of some 20μ , while the refringent granule was a conspicuous object. On the following day, the movements were less energetic; on many occasions one filament appeared to be protruded and two retracted or vice versa; sometimes two filaments seemed to be protruded and retracted, while finally, the rounded but empty sheath was occasionally visible in front of the anterior extremity.

My final opinion is that the prepuc and filament described by Manson do not exist in *Ml. papionis*, such appearances being mere optical illusions occasioned

by the rapid movements of the larva in a sheath of low visibility. The refringent granule, together with the clear area behind it, would appear to be morphological entities, but at the present time it seems more reasonable to interpret these appearances as indicating the commencing formation of the buccal orifice and anterior part of the digestive tube, than to postulate the existence of a kind of sac from which an apparently useless filament is capable of being protruded.

The excretory and genital cells. According to the hypotheses of Rodenwalt and Fülleborn, the excretory cell gives rise to the excretory apparatus of the adult, while the first genital cell is the embryonic representative of the future genital system. Looss suggests that the accessory genital cells may give rise to the rectal ligaments and their adnexa. They and other workers assume the cellular structure of these formations to have been completely demonstrated, so before attempting to criticise the reasonableness of their belief, I will first of all describe these so-called cells as they exist in *Ml. papionis*.

Description of the excretory and genital cells. In wet preparations stained with Giemsa's solution and differentiated with alcohol, these formations are present in a considerable proportion of larvae. The excretory cell (Plate VIII, fig. 2, *Ex.C.*) is blue in colour, more or less oval in shape, and contains a darkly staining homogeneous central portion surrounded by a lighter area—the nucleus and nucleolus of Fülleborn. The blue outline is often deficient anteriorly, its lateral portions being continued forwards as two blue streaks which end at the periphery of the pore-chamber; when complete, this outline is usually continued in a forward direction as a single streak. Sometimes the cell appears to be double; frequently it is not individualised at all, its place being taken by one or more dark-blue streaks. Whether the cell is visible or not, these streaks are often continued in a posterior direction as far back as the beginning of the central viscus (Plate VIII, fig. 2, *str.*).

The first genital cell (Plate VIII, fig. 2, *G.C.*) is larger and as a rule more distinct than the excretory, measuring from 10 to 15 μ in length and up to 5 μ in breadth, but its shape is more variable, the ends appearing either rounded or square-cut. Often the outline of the cell is deficient posteriorly, in which case its lateral boundaries may be continued for some distance in the direction of the anal pore as an irregular double streak; when present, this outline is frequently brought into connection with the anal pore-chamber by an irregular series of blue streaks. Along these streaks the subsidiary genital cells (Plate VIII, fig. 2, *S.G.C.*) may be seen, but their individualisation is exceptional; they never exceed three in number, are quite small with a rounded outline, and contain a central darkly staining spot (Plate VIII, fig. 2, *S.G.C.*). In wet preparations stained for half an hour with carbolmethylgreen-pyronin and differentiated with alcohol (Plate VIII, fig. 4), the subcuticular and body nuclei are stained green while the excretory and genital cells are outlined in red; the central spots are stained an intense red by the pyronin and this colouration is often so marked in the case of the first genital cell as

to make its central spot the most prominent object in the whole larva; at this stage of differentiation, red streaks are to be seen between the cells and their respective pores. If the preparation be a little less differentiated, the red streaks reach from pore to pore but are absent in front of the excretory and behind the anal pore. If still less differentiated, the pore-chambers and central viscus take the red stain, and finally the pyronin predominates everywhere.

These formations are usually well shown after treatment with iron haematoxylin (Plate VIII, fig. 3, *Ex.C.*, *G.C.*). The central spot appears as a black, homogeneous body in a more lightly siderophile frame, the shape of which varies as previously described; no streaks are to be seen, while the cell outline is often feebly or not at all indicated. In dry blood-films stained by the panoptic method or with Giemsa, these cells are invisible; whilst in films stained by the panoptic method immediately after drying, the protoplasm between the body nuclei is coloured a dark green or blue in the neighbourhood of the pore-chambers (Plate VIII, fig. 1), while the pore-chambers themselves may be lightly tinted in the same way. After "vital" staining with azur II, the pore-chambers are coloured first, the excretory and genital cells next, and last of all the subcuticular and body nuclei.

Conclusions. Although I have followed the terminology of Rodenwalt in referring to the excretory and genital cells, I have only done so in order to facilitate their description. I am unconvinced as to their cellular structure for the following reasons:

- (1) These so-called cells are frequently not individualised at all.
- (2) When present, their outline may either be double or incomplete.
- (3) I was unable to demonstrate the presence of chromatin or anything resembling a true nucleus.

An interpretation of these formations. The following facts would seem to indicate that—whatever the ultimate destiny of these formations may be—they function temporarily as part of an excretory apparatus, the products of excretion being eliminated by the pores.

(1) The presence of streaks, extending from the neighbourhood of the central viscus to the pore-chambers, along the course of which the so-called excretory and genital cells may be developed, is especially well shown on differentiating preparations fixed wet and stained with carbolmethylgreen-pyronin; moreover these formations are the last to lose the pyronin.

(2) After vital staining with azur II, the pore-chambers, together with the excretory and genital areas, are coloured before the subcuticular and body nuclei.

The central viscus. The central viscus is frequently stated to represent the remains of the vitellus and to consist of reserve material, but it does not exist in coiled embryos, and is small or absent during the uncoiling process; moreover, it is frequently small or invisible in larvae from the blood. In the

presence of these facts I am unable to support the view that the central viscus represents the remains of the vitellus.

Why this structure should be well developed in one species and poorly developed in another, is unknown. It is unknown whether the granules which enter into its composition have anything to do with the subsequent formation of the gut or not: whether they appear and disappear in a cyclical manner or whether—once formed—they persist until the larva dies or is taken up by the intermediate host, is also unknown. Under these circumstances it would seem premature to refer to these granules as consisting of reserve material.

III. General Biological Investigations.

A. VARIOUS PARASITES OBSERVED IN *PAPIO CYNOCEPHALUS*.

P. cynocephalus is a robust animal, the adult male weighing up to sixty pounds or more. *Loa papionis* was present in ten, *Microloa papionis* in twelve of the fifty-five animals examined; but no other members of the family—either adults or larvae—were discovered. At the autopsy, the internal organs always appeared healthy, although various parasites were frequently present in considerable numbers. The following were noticed at one time or another, but their identity or otherwise with known species has not yet been determined:

Plathelminths. A cestode.

Nemathelminths. *Physaloptera*, *Agchylostoma*, *Oxyurus* and *Trichocephalus*; also a small unidentified nematode from an omental cyst.

Arthropoda. The mesentery was often heavily infected with larval *Porocephalus*.

Protozoa. Protozoa were not especially looked for.

No *Plasmodia* were seen, but a small *Trypanosoma* was once noticed in a fresh preparation. It measured about 10μ in length, while its maximum breadth was 1.5μ . The undulating membrane was but feebly indicated and no projecting flagellum was seen; the macronucleus was a conspicuous object, being situated about the middle of the trypanosome.

An unsuccessful attempt was made to cultivate the organism in Novy and McNeil's medium. The inoculation of a small portion of clot under the skin of a mouse was also tried, but with negative results. Unfortunately the baboon had been destroyed before I thought of examining its lymphatic glands.

B. EOSINOPHILIA IN *P. CYNOCEPHALUS*.

Even in the most extensively infested animals a distinct degree of eosinophilia was never encountered, the differential leucocyte counts charted in Table V being typical.

Table V.

*Differential leucocyte count in six baboons examined shortly before operation.
200 cells counted.*

Findings at autopsy	Polymorphs	Lymphocytes	Hyalines	Eosinophiles	Basophiles
No. 1. Marked <i>Loa</i> infection; no other parasites found	80.5	13.0	6.0	0.5	0.0
No. 2. <i>Loa</i> , <i>Agchylostoma</i> , <i>Physaloptera</i> , <i>Oxyurus</i> and <i>Trichocephalus</i> ...	54.0	31.0	12.0	2.0	1.0
No. 3. Slight <i>Loa</i> infection; many <i>Physaloptera</i>	62.5	24.5	11.0	1.5	0.0
No. 4. Many <i>Physaloptera</i> and also a <i>Taenia</i>	42.0	35.5	15.0	7.5	0.0
No. 5. Mesentery heavily infected with larval <i>Porocephalus</i>	48.0	37.5	9.0	5.0	0.5
No. 6. No parasites found	42.0	40.8	15.0	2.0	1.0

C. A COMPARISON OF CERTAIN BIOLOGICAL PHENOMENA IN THE DEVELOPMENT OF *LOA PAPIONIS* AND *L. LOA*.

A short account of Loa loa. This parasite has been known in equatorial Africa for hundreds of years. The adult worm is usually found under the skin, where its presence may give rise to localised and temporary oedemas—the so-called “Calabar swellings”; it is sometimes met with in the eye—more especially under the conjunctiva—and in these situations is also liable to cause local symptoms. After the death of the host it is usually met with under the skin, but has been occasionally discovered in the pericardial and peritoneal cavities; its presence in the cranial cavity has also been reported by Brunetière (1913) who suggests the possibility of its having been the exciting cause of an attack of hemiplegia. Infection in man is usually associated with a considerable degree of eosinophilia, 40 to 50 per cent. of eosinophiles being quite common. As a rule, the larvae are only found in the peripheral circulation during the daytime, but this diurnal periodicity may be lost under certain abnormal conditions. Three to four years are supposed to elapse between infection and the appearance of the larvae in the blood of the definitive host. The larvae have been shown by Leiper (1912) to undergo a further stage of development in the salivary glands of certain West African tabanid flies belonging to the genus *Chrysops*; these flies bite exclusively during the daytime.

The distribution of Loa papionis in P. cynocephalus. Adult *L. papionis* were found in ten out of fifty-five autopsies. Their favourite haunts were the subcutaneous tissues overlying the serratus magnus, the trapezius and the upper part of the latissimus dorsi muscles, together with the axillary folds and the region of the neck; I seldom noticed them in the inguinal or pubic regions or under the skin of the limbs, and never succeeded in finding them either in or near the eyes or in the serous cavities. However M. Bruck, who had previously performed several hundred autopsies on these animals with a view to excluding the presence of tuberculosis, etc., showed me the parasites he had found and gave me the filariae for identification. Six out of the seven

were Loas; three of them were found in the subcutaneous tissues, while the remaining three came from the pericardium, the peritoneal cavity and the upper surface of the diaphragm respectively; the seventh was found between the layers of the mesentery and did not belong to the genus *Loa*.

The distribution of Microloa papionis. When larvae were discovered in the peripheral circulation of the host during life, they were invariably present at night¹; they were less numerous or absent in the daytime. (Table VI.)

In five animals in which the peripheral blood had been examined with negative results during life, larvae were found in the heart's blood at the autopsy. (Table VI.)

In another case, no larvae were found either during life or at the autopsy: nevertheless Dr Quéry exposed an adult *Loa* under the skin of the neck when bleeding the animal, while two more were found at the autopsy after a prolonged search. No larvae were discovered in citrated and centrifuged blood from the heart and carotid artery, and smears made from the lungs and other organs were also negative. The worms proved on examination to be full grown but unimpregnated females. These facts are interesting in that they possibly explain a point raised by Low (1913) as to the "occasional absence of larvae from human blood in cases where adult females are known to be present in the tissues."

Table VI.

The periodicity of Microloa papionis.

	Peripheral blood during life		Carotid (at operation)	Heart's blood (at autopsy)
	Day	Night		
No. 14	○*	—*	+	+
No. 17	○	+	+	+
No. 18	○	+	○	○
No. 24	+ scanty	+	+	+
No. 28	○	○	+	+
No. 31	○	—	+	+
No. 36	—	○	+	—
No. 38	+	+	+	+
No. 39	○	+	+	+
No. 42	○	—	+	+
No. 48	+ scanty	+	+	—
No. 55	+ scanty	+	—	—
Percentage of positive results	36.3	77.7	90.9	88.8

* + signs denote that *Microloa* were found, ○ signs denote negative results, and — signs indicate that no examination was made.

At the autopsy, the larvae were chiefly concentrated in the heart, lungs and large pulmonary vessels: it is possible, however, that the preliminary bleeding of the host affected their distribution to some extent. In the two animals in which a careful search was made, the distribution of the larvae was

¹ The animals were usually examined between midnight and 1 A.M.

identical (Table VII); on the whole they were most numerous in blood from the heart.

Table VII.

Distribution of larvae at autopsy—two cases.

Site	Larvae
Blood from auricles and ventricles	Numerous
Blood from large pulmonary vessels	Present in fair numbers
Smears from cut surface of lung	„ „
Blood from aorta	Scanty
Blood from inferior vena cava	„
Blood from superficial vein of forearm	Very scanty
Smears from cut surface of liver, spleen and kidney	Absent

*Questions arising from the absence of diurnal periodicity in
Papio cynocephalus.*

The liability of man to infection. Apart from cases of sleeping sickness and altered sleeping habits, the diurnal periodicity of *Ml. loa* is so marked that human infection with *Ml. papionis* would seem to be improbable.

The nature of the intermediate host. The nocturnal periodicity of the larvae suggests the transmission of the infection by a night-biting intermediate host. I had started experiments with *Anopheles maculipennis*, but they were interrupted by the war before any conclusions were reached.

The development of Loa loa and Loa papionis after inoculation by the intermediate host. *L. loa* is certainly a long-lived parasite, for Manson (1912) quotes a case in which the worm was extracted from the eye thirteen years after the patient had left Africa. All observers are agreed that its development must be slow, for in villages where nearly all the adults are carriers, larvae are absent in the blood of the children, although their chances of infection are presumably identical. Yet in spite of these facts, we have no detailed information as to the parts of the body inhabited by the parasite before reaching sexual maturity. I had no opportunity of examining young baboons, but never found immature Loas in the adult animals, although I was able to make detailed autopsies under the best conditions. Certainly the female Loas had not always been fertilized, but such females were approximately the same size as the rest and seemed fully developed in every respect. I shall refer to this matter again when considering the subject of immunity.

The liability of Loa papionis to disease. I can find no reference to diseased Loas in the literature, but they are undoubtedly liable to parasitic infections, as the following example shows: Three Loas were removed at the autopsy of a baboon in which few larvae were found during life. The peculiar dead-white appearance of one of them attracting attention, all three were kept for further examination. The opaque worm was fully as active as the other two, and all three were alive on the following day; nevertheless the opacity was due to disease. After fixation and clearing, the cuticle was still very indistinct

and covered with a fine powder which, on examination with the oil immersion, resolved itself into large numbers of small bacteria measuring about 3 by 1 μ ; these were, for the most part, arranged end to end in short rows and covered a large portion of the surface of the body. The general body-cavity was packed in many places with granular débris, while the mid-gut was in a state of atony, its posterior half measuring in places more than 300 μ in diameter instead of the normal 120 to 180 μ .

The other two were also slightly infected in the same way. An attempt was made to cultivate these organisms in broth and on agar slopes, but the media remained sterile.

The absorption and calcification of dead Loas by the host. The calcification of dead Loas has been occasionally reported in autopsies on the human subject. In *Papio cynocephalus*, from 5 to 10 per cent. of the Loas found at autopsy were dead and calcified: the greatest number ever found in one baboon was forty-three, of which three had undergone this process. Whether they can be absorbed without undergoing calcification, I am unable to say.

D. THE INFLUENCE OF THESE PARASITES ON THEIR RESPECTIVE HOSTS.

Traumatic action. In the living worms of both sexes, the mid-gut often showed through the cuticle as a bright-red streak owing to recent blood absorption; this does not seem to have been noted for *L. loa*.

Toxic action. Although no localised oedemas comparable to "Calabar swellings" were ever noticed, I am inclined to think that, in moderately or heavily infected animals, the subcutaneous fat was diminished in quantity and paler than normal. An investigation of the possible toxicity of *L. papionis* for other animals seemed therefore desirable, and the following experiments were made on guineapigs.

Experiments. Three healthy adult male guineapigs, whose blood had been examined on many occasions during the preceding three months, were chosen.

Two male and two female Loas were introduced under the skin of the back of guineapig no. I, but the stitches not being sufficiently close together, two of them escaped shortly afterwards. Two males and three females were insinuated under the skin of the back of guineapig no. II, care being taken to remedy the defect in technique. Guineapig no. III served as a control. A week later, no. II was found to be greatly emaciated, while the other two remained fat and healthy. No. II had always showed a slight lymphocytosis, and this had now become accentuated; but the percentage of eosinophiles was not increased. The animal died on the ninth day, having lost sixty grammes in weight during the last 24 hours. At the autopsy, which took place about 12 hours after death, four Loas were found out of the five which had been originally introduced. One female was discovered in the left lumbar region between the peritoneum and the muscles of the back, a second beneath the

skin of the anterior abdominal wall, while the third was lying dead in the region of the wound; of the two males, only one was found and this with some difficulty—it had hidden itself in the connective tissue between the right hamstring muscles. All fat had disappeared from the body, otherwise the organs seemed healthy. No larvae were noticed either in the peripheral blood, the heart's blood, or in smears from the various organs, but two were seen in a smear made from the connective tissue in which a female had been found; eosinophiles were not a marked feature of the preparation. The three living Loas removed at the autopsy of guineapig no. II were left for two hours in normal saline and then introduced into another guineapig.

I was unable to continue these experiments owing to the war and killed the animal the day after. All three worms were found *in situ* and their movements were as vigorous as when taken from the baboon ten days earlier. Guineapigs nos. I and III were killed at the same time. No Loas were found in no. I in spite of a careful search; they had almost certainly escaped from the wound shortly after the operation. Both animals were healthy.

Conclusions. Although the possibility of guineapig no. II having died of some undiagnosed infection was not absolutely excluded, it seems more reasonable to suppose its death to have been occasioned by the presence of the Loas.

The reaction of the host. The following facts point to the possession of a considerable, though variable, degree of immunity against *Loa* infection by the adult baboon.

(a) Out of the fifty-five baboons examined only thirteen (23·6 per cent.) were found to be infected.

(b) The degree of infection was very variable in the parasitised animals. Neither males nor larvae were present in one case; in another, forty-three Loas were found, while the blood was swarming with larvae.

(c) The apparent absence of immature Loas in the tissues of infected animals points to the existence of a high degree of immunity against reinfection in the adults.

E. THE TREATMENT OF FILARIAL DISEASE.

The mechanism of immunity in filariasis and the possibility of manufacturing prophylactic and curative antisera against these infections are subjects which have as yet been very imperfectly investigated in man. Yet the apparent toxicity of Loas for guineapigs, together with the undoubted immunity possessed by adult baboons against *Loa* infection, point to the production of toxin by the Loas and antibody by the host. Moreover it is possible that such antibody exists in sufficient quantity to effect a cure of *Loa* infection when baboon serum is introduced into human beings; for Quéry (1918) has recently reported the case of a patient who, after receiving a course of antisyphilitic serum, wrote to the effect that the *Loa* infection from which he had suffered up to the time of the injections, had been apparently cured by them.

Taking the known facts into consideration, it would seem as though the production of an efficient anti-*Loa* serum were possible. The subject requires investigation.

SUMMARY.

Papio cynocephalus from French Guinea frequently harbours *Loa papionis* n.sp. The intermediate host is unknown. The larvae, in contradistinction to those of the human parasite *Loa loa*, show no diurnal periodicity.

The occurrence of bacterial disease in adult *L. papionis* would seem to be a novelty; at any rate I could find no mention of diseased filariae in the literature.

In so far as the biology of *Microloa loa* is concerned, I am inclined to doubt the correctness of the opinions expressed by Rodenwalt and Fülleborn concerning the structures they term "executory and genital cells": the current hypotheses concerning the significance of these structures and of the "central viscus," clearly need revision. Manson's "buccal apparatus" would seem to be nothing more than an optical illusion, while the "neck" or "shoulder" described by the older observers as existing in adult *L. loa* after fixation, was evidently due to imperfect technique.

I have drawn attention to the desirability of standardising the technicalities which are such essential preliminaries to the accurate mensuration of microfilariae, and have suggested a method which has certain advantages over those at present in use.

Concerning the pathology of *Loa* infection, evidence both of the traumatic and toxic action of *L. papionis* on baboons, was obtained; moreover these parasites seemed definitely toxic for guineapigs, although the single experiment performed needs confirmation. The available evidence favours the supposition that adult baboons from an infected area possess a high degree of immunity both against *L. papionis* toxæmia and against reinfection by these parasites; but whether their serum is of therapeutic value for human beings infected with *L. loa*, requires further investigation.

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EXPLANATION OF PLATES VIII AND IX.

PLATE VIII.

Larvae of *Loa papionis* stained by various methods; also the ovum immediately after fertilization.

- Fig. 1. The general structure of the larva is well shown but wet fixation is necessary for the demonstration of the excretory and genital cells. From blood-film stained by Pappenheim's panoptic method.
- Fig. 2. Showing the streaks which connect the central viscus with the pore-chambers; also the excretory and genital cells which frequently develop along the course of these streaks. From thick blood-film fixed wet in 70 per cent. alcohol at 60° C. and stained with Giemsa's solution.
- Fig. 3. Showing excretory and genital cells, pore-chambers, central viscus, nuclei of subcuticular cells, and body nuclei. From thick film fixed wet and coloured by Heidenhain's iron haematoxylin method.
- Fig. 4. Owing to the absence of the central viscus, the reduction of the body nuclei in this region is very obvious. From thick film fixed wet and stained with carbolmethylgreen-pyronin.
- Figs. 5 and 6. Showing the comma-shaped structures described by Fülleborn as existing at either extremity in *M. loa*. Fig. 5. From dry film fixed in absolute alcohol and stained with Giemsa's solution. Fig. 6. From dry film stained with Giemsa's solution without preliminary fixation.
- Fig. 7. Egg from *Loa papionis* immediately after fertilization. Showing spermatozoon, and so-called egg membrane which does not exist before the entrance of the spermatozoon. From fresh material examined in a 1 in 3000 solution of Azur II in 0.9 per cent. saline.

Fig. 1 × by about 1200.

Figs. 2-6 × by about 1000.

Fig. 7 × by 1000.



Gilberte R. Blanchard, del.

Cambridge University Press

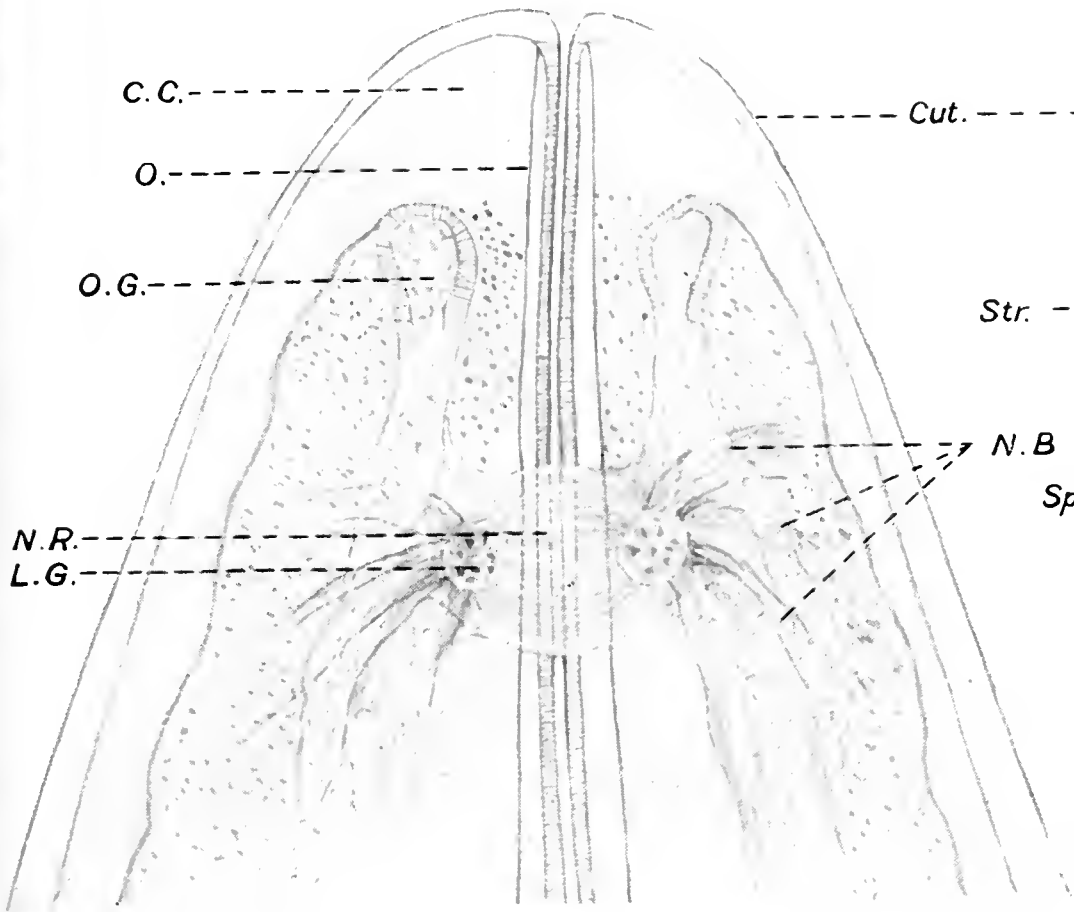


Fig. 1

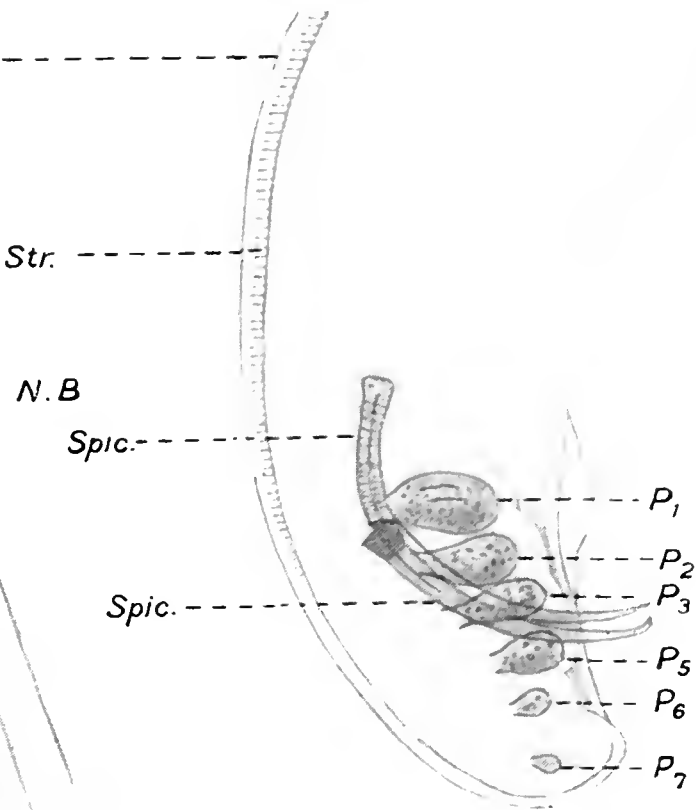


Fig. 2

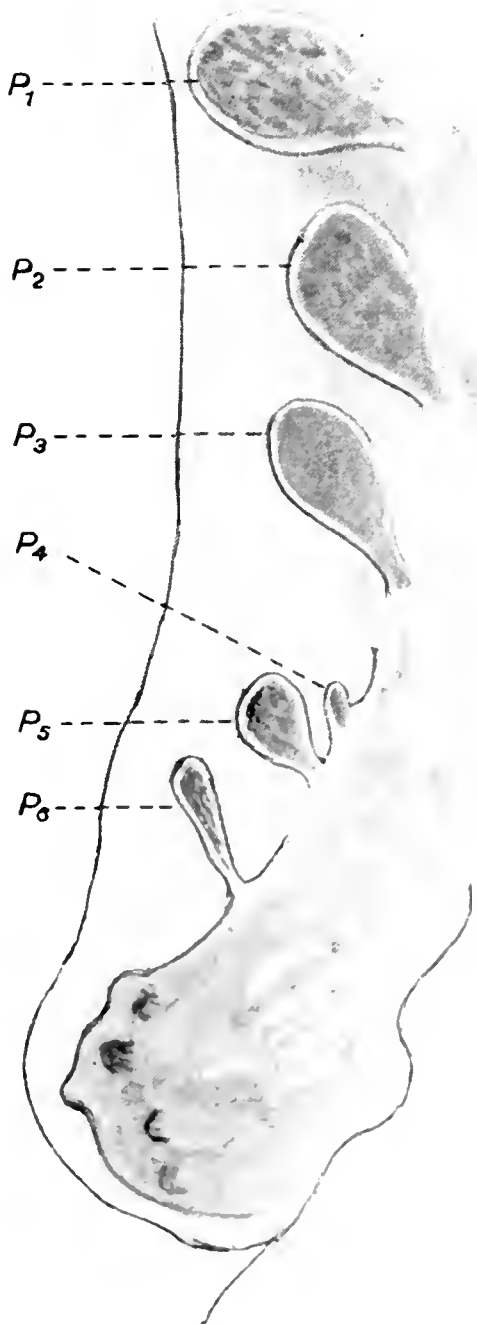


Fig. 3

KEY TO LETTERING.

A.P. = position of anal pore; *A.P.C.* = anal pore-chamber; *C.B.* = comma-shaped bodies; *C.V.* = central viscus; *E.M.* = egg membrane; *Ex.C.* = excretory cell; *Ex.P.* = position of excretory pore; *Ex.p.c.* = excretory pore-chamber; *G.C.* = genital cell; *N.R.* = nerve ring; *N.S.C.* = nuclei of subcuticular cells; *S.G.C.* = subsidiary genital cells; *S.P.* = spermatozoon; *Str.* = streaks connecting excretory cell with central viscus; *T.S.* = tail spot.

PLATE IX.

Loa papionis after fixing in 70 per cent. alcohol at 60° C. and clearing in lactophenol.

Fig. 1. Anterior extremity of ♀. Showing cephalic cone, oesophagus, nerve ring and oesophageal gland.

Fig. 2. Lateral view of posterior extremity of ♂. Showing spicules and six out of the seven genital papillae on the right side; the fourth papilla is invisible.

Fig. 3. One-half of posterior extremity of ♂ after removal of spicules and hemisection. The fourth genital papilla is now visible.

Figs. 1 and 2 × by about 180.

KEY TO LETTERING.

C.C. = cephalic cone; *Cut.* = cuticle; *L.G.* = lateral ganglion; *N.B.* = nerve bundles; *N.R.* = nerve ring; *O.* = oesophagus; *O.G.* = oesophageal gland; *P_{1,2}*, etc. = first, second genital papillae, etc.; *Spic.* = spicule; *Str.* = striae.

ON FAHRENHOLZ'S PURPORTED NEW SPECIES, SUB-SPECIES AND VARIETIES OF *PEDICULUS*.

A CRITICISM OF METHODS EMPLOYED IN DESCRIBING ANOPLURA.

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(With 2 Charts.)

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INTRODUCTION.

THE object of this paper is to draw attention to certain grave sources of error underlying the present mode of differentiating Anoplura¹ and more especially species of the sub-order Siphunculata. I am unable to say if the criticisms herewith advanced apply equally to the methods employed in differentiating members of the sub-order Mallophaga, because I am not familiar with the latter group, but the two sub-orders mentioned are in many respects so closely allied that it appears difficult to avoid the suspicion that the considerations herein contained may apply equally in both cases.

Fahrenholz is a recognized authority on Anoplura, he has dealt with the group in *Das Thierreich*, and consequently is in a position to mislead others who may concern themselves with the study of these ectoparasites.

¹ The order Anoplura Leach, 1817, includes two sub-orders: Mallophaga and Siphunculata (see Nuttall, *Parasitology*, XI. 332).

Until recently, owing to the war, most of Fahrenholz's publications herein referred to were rendered inaccessible to me, otherwise their consideration would necessarily have been included in my previous paper (*Parasitology*, XI. 329-346).

The subject-matter that follows has been arranged by me in three sections dealing with (1) Fahrenholz's descriptions of supposedly new species, etc., (2) my detailed criticism thereon, and (3) my conclusions therefrom in respect to the synonymy of *Pediculus humanus*.

I.

FAHRENHOLZ'S EVIDENCE IN SUPPORT OF THE VALIDITY OF HIS SPECIES, SUB-SPECIES AND VARIETIES OF *PEDICULUS*.

In considering the forms described by Fahrenholz, it appears convenient to class them in two groups:

(a) Lice derived from man.

Pediculus humanus race *corporis* (mihi).

P. nigritarum Fabricius 1805. Regarded by Fahrenholz as a variety (1915) and subsequently as a sub-species (1916).

P. humanus chinensis Fahrenholz 1916. A sub-species.

P. humanus marginatus (Fahrenholz 1915) Fahrenholz 1916. A sub-species.

"*P. humanus humanus* L." of Fahrenholz 1917. A sub-species.

Pediculus humanus race *capitis* (mihi).

P. capitis angustus (Fahrenholz 1915) Fahrenholz 1916. A sub-species.

P. capitis maculatus (Fahrenholz 1915) Fahrenholz 1916. A sub-species.

"*P. capitis capitis* de Geer" of Fahrenholz 1917. A sub-species.

(b) Lice derived from apes and monkeys.

P. schäffi Fahrenholz 1910.

P. oblongus Fahrenholz 1913, subsequently renamed *assimilis* (see below).

P. friedenthali Fahrenholz 1916.

P. lobatus Fahrenholz 1916.

P. assimilis Fahrenholz 1919 (= *oblongus* renamed).

(a) Lice derived from man.

In support of his contention that the lice occurring on various races of man constitute distinct sub-species, Fahrenholz (1915, p. 591 *et seq.*) cites Fabricius (1805), Olfers (1816), Denny (1842), Pouchet (1841, pp. 204-5,

quoted by Küchenmeister, 1855, p. 438), O. Fabricius (from Schiödte, 1854), Wallace (1853), Küchenmeister (1855), Murray (1861), and Lumholz (1892).

I have been able to verify the references to Fabricius, Denny, Schiödte and Wallace, those to Pouchet and Lumholz were only accessible in other editions of their works, whilst Olfers and Küchenmeister have proved inaccessible to me.

According to Küchenmeister (1855), Pouchet (1841) regarded the lice of negroes as a distinct species, but I find that Pouchet (1832, p. 412) makes no such statement. Fahrenholz cites Wallace as believing that Amazon Indian lice differ from those of Europeans, but Wallace (1853, p. 244) expresses himself cautiously, stating only that they are "probably a distinct species from that of our own country." Küchenmeister (1855, p. 428, Pl. IX, figs. 9-15) examined *nits* on the mummies of a New Zealander and Peruvian, and measured the claws of the therein contained louse embryos, finding as he supposed that they differed from those of European lice. Fahrenholz (1915, p. 593) remarks that to Küchenmeister undoubtedly belongs the credit "*auf den tatsächlich vorhandenen Längenunterschied in den Krallen der Läuse verschiedener Menschenrassen hingewiesen zu haben*¹."

Schiödte (1854, p. 154) merely *supposed* that the Greenlander's lice, referred to by O. Fabricius, probably constituted a distinct species like those on negroes, but O. Fabricius (1780, p. 215) makes no mention, I find, of these lice being different from those on Europeans.

Lumholz (1889, p. 117, English edition) states that the head and body of most Australian natives are heavily infested with rather large dark lice that are "quite different from the common *Pediculus capitis*"; Lumholz did not become infested with the Australians' lice though they were dropped about in plenty.

We may now consider in some detail the characters which Fahrenholz, in his various publications, ascribes to the forms he distinguishes:

***Pediculus nigritarum* Fabricius 1805.**

Synonyms:

P. nigritarum Fabricius 1805, p. 340.

P. nigrescens Olfers 1816 (*fide* Fahrenholz).

P. corporis var. *nigritarum* (Fabricius 1805) Fahrenholz 1915, p. 597, fig. 1 (sternal plate).

P. corporis nigritarum (Fabricius 1805) Fahrenholz, VII. 1916, p. 270. Designated as a sub-species for no apparent reason (*vide supra*).

¹ The italicized passages in the quotations from Fahrenholz throughout this paper are printed in spaced type by that author.

N.B. The sign ● introduced by me into citations from Fahrenholz indicates that I challenge the value of the statement he makes. Refer to my adverse criticisms as to the validity of the characters he gives in his diagnoses. See pp. 143 *et seq.*

In my previous papers, whilst I quoted Fabricius (1775 and 1794), I omitted to cite his publication of 1805 to which my attention has since been drawn by a reference of Fahrenholz's (1915). On consulting Fabricius (1805), I find that he describes lice from negroes as *P. nigritarum* n.sp., distinct from *P. humanus*:

Habitat in Nigritarum corpore. Dom. Smidt. Mus. Dom. Lund.

Paullo minor *P. humano*. Caput magnum, planum, laeve, triangulum, antice, subbidum, atrum. Corpus subrugosum, atrum, immaculatum.

Therefore, as Fahrenholz notes, Fabricius appears to be the first to have regarded the lice of negroes and Europeans as distinct species. Denny (1842, p. 15) states that Latreille "designated" *P. nigritarum* as a species; Fahrenholz, however, finds no record of Latreille having created this supposed species.

Olfers (1816, Pars I, p. 81, cited by Fahrenholz 1915, p. 591) regarded lice from Ethiopians as a distinct species which he named *P. nigrescens*. Fahrenholz is almost right in regarding *nigrescens* as "doubtless" a synonym of *nigritarum*.

Fahrenholz degrades Fabricius's *nigritarum* to a variety of *corporis* and ventures to describe the variety from a single ♀ which he assumed represents the form named by Fabricius. Fahrenholz states that his specimen (from the Tana region) represents a distinct variety differing from negro head-lice as follows: Antennae not linear like European body-lice, but of inverted pear-shape ("umgekehrt birnförmig")●; sternum present●; the last abdominal segment bears two brown plates dorsally (as in negro head-lice)●.

***P. humanus chinensis* Fahrenholz 1916.**

A sub-species.

Fahrenholz (x. 1916, p. 87) states of this form that it occurs on Chinese, is "*distinctly larger*"● than "*P. humanus marginatus*" (*vide infra*), and has finely dentate claws.● Of the ♂ he writes: "*schwachen Querplatten auf dem Abdomen*●, *aber auch mit gut entwickelter Genitalplatte*●. *Sternum vorhanden*●. *Allgemeinfärbung bräunlich-gelb (in balsam)*●.—*Lebt auf Chinesen.*"

Fahrenholz (1917, pp. 2, 6 and text-fig., reprint) states that he identified specimens of this form at the Hamburg Museum¹. They came from Fokim, China, and he assumes that the host was a Chinese. He comments that they are "distinctly different from those on Japanese" and gives elaborate measurements and a figure of the ♀ sternum with two hairs anteriorly but no "holes"●.

¹ The lot also contained some examples of *capitis* "die vielleicht ebenfalls einer neuen Unterart angehören" (!).

***P. humanus marginatus* (Fahrenholz 1915) Fahrenholz 1916.**

A sub-species.

Synonyms:

“*P. corporis* de Geer var. *marginatus* n.var.” Fahrenholz 1915, p. 599.*P. humanus marginatus* Fahrenholz VII. 1916, p. 270, and x. 1916, p. 87.

Lice from Japanese, which Fahrenholz believes to be identical with the form described by Murray (1861) as having no spine on leg I. Fahrenholz found, however, that a spine is present and was doubtless lost in the preparation of Murray's specimen. When mounted in balsam, the lice appear pale yellow●, black-brown at the sides of the abdomen●, and in places on the head●. The sternum is “totally absent●.” The ♂ shows no ventral plates●; whilst of the dorsal bands only the anterior one of each segment is well developed, the posterior one being scarcely visible●; *these bands do not occur in European body-lice*●, compared to which they are somewhat smaller●. In his two papers of 1916, Fahrenholz raises his variety to the rank of a sub-species, and adds (x. 1916, p. 87): It is much smaller than the European louse● and has claw I dentate●. “Am Abdominalrande schwarzbraune Chitinleisten●. *Sternum fehlt vollkommen*●, ebenso beim ♂ die Genitalplatte●. ♂ im Gegensatz zur Europäerlaus mit *Querplatten* auf dem Abdomen●. Allgemeinfärbung gelblich●.—Lebt auf Japanern.”

“*P. humanus humanus* L.” of Fahrenholz 1917.

A sub-species.

This new form of terminology, for which Linnaeus need not be held responsible, is applied by Fahrenholz (1917, p. 1, reprint) to specimens in the Hamburg Museum labelled “Negerläuse aus Sansibar.” Fahrenholz greatly doubts their recorded origin because they are similar to European body-lice, which, as a piece of special pleading, is difficult to match. He writes: “Der Wirt ist mir sehr zweifelhaft, da die Individuen keine Abweichungen von typischen Europäerläusen aufweisen●.”

***P. capitis angustus* (Fahrenholz 1915) Fahrenholz 1916.**

A sub-species.

Synonym:

“*P. capitis* de Geer var. *angustus* n.var.” Fahrenholz 1915, pp. 597–8, Pl. XXI, fig. 1 (photo of ♀), text-fig. 2 (♂ abdomen in dorsal aspect).

This form, found on Japanese, is described from balsam-mounted specimens (♂ ♀), Fahrenholz stating that the colour is distinctive●. The ♂ shows dorsal bands●, brown sternum●, and genital plate● which are absent in European lice●; claw I is longer and more curved than in the ♀, and finely toothed irregularities only occur in place of teeth●.

In his two papers of 1916 Fahrenholz raises his variety to the rank of sub-species, terming it *P. capitis angustus* in the first paper (vii. 1916, p. 270), and *P. corporis angustus* in the second (x. 1916, p. 88, *corporis* being obviously printed in error for *capitis*). To the earlier description he adds that *angustus* scarcely attains the size of European *capitis*●, but is *much narrower*, appearing much slenderer●. Claw I is very long and dentate●. “Allgemeinfärbung hellgelb●; Chitinisierung gut entwickelt; Randplatten des Abdomens tief-schwarz●. Querplatten des Abdomens beim ♂ sehr deutlich●; desgleichen die Genitalplatte●. Sternum vorhanden●, aber Ränder undeutlich.”

Fahrenholz (1917, p. 2, reprint) refers to specimens of *angustus* at the Hamburg Museum.

***Pediculus capitis maculatus* (Fahrenholz 1915) Fahrenholz 1916.**

A sub-species.

Synonym:

“*Pediculus capitis* de Geer var. *maculatus* n.var.” Fahrenholz 1915, p. 598, Pl. XXI, fig. 2 (photo of ♀), fig. 3 (photo of ♂), text-figs. 3, 4 (♂ venter, ♀ sternum).

This form, emanating from Cameroon negroes, is described from balsam-mounted specimens (♂ ♀). The lice have a light yellow colour● in which they differ at once from lice on Japanese. Pleurae broader than in Japanese lice●. ♂ with dorsal bands dark brown●, sternum●, genital plate● and ventral band clearly defined●, four holes in the sternum bearing hairs●. ♀ with two brown spots dorsally on last abdominal segment● (as in *Haematopinus*); sternum clearly defined●, with two holes anteriorly bearing hairs●.

In his papers of vii. 1916, p. 271; x. 1916, p. 88, and 1917, p. 2 (reprint), he raises the variety to the rank of a sub-species, stating (x. 1916, p. 88) that the general form is distinctive, being shorter than “*P. capitis angustus*” (*vide supra*)●; whilst the ♀ is “*broader than European capitis*●.” “Gut unterschieden durch hellbraune Grundfärbung●¹. Chitinisierung äusserst kräftig; vordere Randplatten des Abdomens verbreitert●. Querplatten auf dem Abdomen des ♂ dunkel braun●, desgleichen die Genitalplatte● und mediane Ventralplatte des II Segments (our ventral band)●. Sternum deutlich gerandet●. Lebt auf Neger (Kamerun); eine etwas abweichende Form auf Hottentoten².”

“*Pediculus capitis capitis* de Geer” of Fahrenholz 1917.

A sub-species.

Term applied by Fahrenholz (1917, p. 2, reprint) to specimens in the Hamburg Museum identified by Fahrenholz as such.

¹ Light yellow in his first description, light brown in his second.

² Another Fahrenholz variety which no doubt will presently be described.

(b) Lice derived from apes and monkeys.

***Pediculus schäffi* Fahrenholz 1910.**

This form was described by Fahrenholz, 1910, p. 57, Pl. III, figs. 2, 6, and text-fig. 1 (eggs); host: *Simia troglodytes*. The species was condemned and "regarded provisionally" for reasons specified by me (*Parasitology*, XI. 336-7) as = *P. humanus* race *schäffi* (Fahr.). I see no reason to change my opinion.

***Pediculus friedenthali* Fahrenholz 1916.**

The author states (x. 1916, p. 88) that this form occurs on *Hylobates mülleri*. He only describes the ♀ which "much resembles the head-louse of man." After dwelling on trivial points of detail he adds:

"Sternum fehlt●...grösste Breite im V Segment●...Letztes Segment des ♀ breiter als lang● so dass die Gonopoden in den Einschnitt desselben hineinragen. Gonopoden sind nach hinten gerichtet und stehen der Form nach in der Mitte zwischen denen von *P. capitis* und *P. humanus*●."

***Pediculus lobatus* Fahrenholz 1916.**

This form was named by Fahrenholz in 1913 (p. 373) but not described, the host being given as *Ateles pan.* Fahrenholz (x. 1916, p. 89) afterwards named the host *Ateles rellerosus* (sic) and described the louse (♂ ♀) as follows:

"Kopf ähnlich dem von *P. capitis*. Vorderkopf an den Seiten je eine dunkelbraune Platte●. Sternum fehlt●; Thorax ohne Borsten●; Abdomen sehr breit, mit tiefen Einschnitten (besonders beim ♀). ♂ mit zweiteiliger Genitalplatte (wie bei *P. capitis maculatus*)●. Letztes Segment des ♀ hat statt Ausschnitt nur einen feinen Schlitz●."

Prior to seeing the foregoing description, I had concluded (x. 1919, pp. 337, 340) that the form probably represents *P. humanus* race *capitis*. The description merely confirms me in this belief. Several lots of lice that I have examined from different species of *Ateles*, cannot, I find, be differentiated from ordinary *capitis*.

***Pediculus assimilis* Fahrenholz 1919.**

Synonym:

P. oblongus Fahrenholz 1916.

This form was named by Fahrenholz in 1913 (p. 373) but not described. He gives the hosts as *Hylobates concolor* (*mülleri*) and *Symphalangus* (*Hylobates*) *syndactylus*. Fahrenholz (x. 1916, p. 88) gives the host as *Hylobates syndactylus* Desm. and describes the ♀ only. Omitting the trivial details he gives, the diagnosis mentions the following supposedly specific characters:

"grösste Breite im VII Segment●...Gonopoden in Form denen voriger Art gleichend; die nach innen gerichteten Spitzen erreichen nicht den Einschnitt● des letzten Segments, das in Länge und Breite gleich ist●."

Fahrenholz (1919, p. 27) renamed the form *assimilis* finding the name *oblongus* preoccupied. This form was regarded by me (x. 1919, pp. 337, 340) as probably *P. humanus* race *capitis*, and I am confirmed in this belief by the foregoing diagnosis which has since become accessible.

II.

EVIDENCE DISPROVING THE VALUE OF THE CHARACTERS WHEREBY FAHRENHOLZ DISTINGUISHES THE BEFORE-MENTIONED FORMS OF *PEDICULUS*.

Of the older authors who have examined lice obtained from different races of man, Murray (1861), whom I have cited elsewhere (II. 1919, p. 206), did not venture to regard such lice as belonging to distinct species. He does not, however, distinguish clearly between head-lice and body-lice in reaching his conclusions. Piaget (1880, p. 623), who regarded *corporis* and *capitis* as separate species, in referring to the latter, fully appreciated its variability, for he wrote:

“Il ne me semble pas qu'il faille attacher beaucoup d'importance à ces différences. En examinant un plus grand nombre d'individus de la même race, il en sera probablement comme de ce que j'ai eu sous les yeux. Quelques individus avaient le côté interne de la griffe dentellé, d'autres entièrement lisse; la couleur passait du gris au jaunâtre. C'était le cas chez les parasites d'Européens et de Malais faisant partie de ma collection. Du reste, je ne vois pas que l'on puisse tirer de ces minimes différences quelque argument pour ou contre l'unité d'origine des races ou espèces humaines.”

Neumann (1910, p. 411), who also quotes the foregoing passage from Piaget, lays stress on the need of examining many adult lice from various races of man before reaching conclusions. Having examined many such specimens, he concluded that “les transitions sont insensibles entre la forme type (européenne) et les diverses formes exotiques et que l'on est porté à les réunir toutes en une espèce unique.” The pigmented chitinous structures which possess great taxonomic value in Anoplura, correspond in lice from negroes “à des renforcements chitineux, incolores ou peu colorés chez le pou de l'Européen. Il en résulte que *P. capitis* est infiniment mieux caractérisé par les spécimens des races noires....”

It is remarkable that Fahrenholz should attach importance to the superficial statements of the earlier authors I have cited on p. 137 and not have taken a warning from the writings of vastly more careful observers like Murray, but especially Piaget and Neumann, whom I have just quoted.

The subject of the relation between *capitis* and *corporis* has already been discussed at length by me (*Parasitology*, XI. pp. 339 *et seq.*) and I see no reason to modify my statements in view of those of Fahrenholz's publications which have since become accessible to me in the original. I was thinking not only of Fahrenholz but also of others when I stated (*Ibid.* p. 333) that “Such

studies require the use of other methods than the usual one of treating specimens with caustic potash and mounting them in balsam." Fahrenholz's purported species of *Pediculus*, i.e. *schäffi*, *lobatus* and *oblongus* (= *assimilis*) were degraded by me (*Ibid.* pp. 334-340), the first to a race of *P. humanus*, whilst the others were merely regarded as probably forms of the variable race *capitis*. Of Fahrenholz's varieties of "*P. capitis*," i.e. *maculatus*, *angustus* and *marginatus* (since raised to the rank of sub-species by that author), I ventured "to assert that these names will not stand and that they will fall into the synonymy of *P. humanus*," also that they "are surely based on faulty observation." Already in an earlier paper (II. 1919, p. 208), referring to the three above-mentioned varieties, I wrote: "Judging from Fahrenholz's other publications to which I shall refer elsewhere (meaning here), this author has also in this instance merely burdened science with three names which will fall into the synonymy of *Pediculus humanus*." That these comments were justified is proved by the following detailed criticism dealing with (a) the measurements, (b) morphology and (c) pigmentation of *Pediculus* and their bearing on the forms which Fahrenholz has attempted to define.

(a) Measurements of *Pediculus*.

There are certain sources of error that cut at the base of all measurements made on lice, and these require consideration because they have been widely ignored: (a) When lice die and dry up, the soft parts of the integument shrivel so that the insects shrink considerably. The antennae become shortened, the hard parts, this being especially evident in *capitis*, becoming partly telescoped into each other. The neck-like portion of the head is retracted into the thorax, the abdomen collapses and may shorten greatly. (b) When dried lice are placed in water their soft parts imbibe the fluid and the body resumes its normal contour, but by placing them afterwards in alcohol whose strength is gradually increased, such previously dried specimens may be made to retain their normal external form. (c) When dried specimens are placed in 10 per cent. caustic potash their bodies imbibe fluid and become swollen to the limit of their capacity if the exoskeleton is uninjured. (d) When fresh or dry caustic-treated specimens are cleaned and mounted in balsam, they frequently collapse again more or less provided extra precautions are not taken; therefore measurements made on balsam-mounted specimens are fallacious in most cases.

When living or well preserved lice are examined, it is evident (e) that the amount of food they have imbibed alters their dimensions and (f) that the number of eggs contained in the body of the female distinctly alters the length and width of the abdomen. Moreover, (g) the head protrudes more from the body of fully fed lice than from those that are unfed or but partly fed.

As an example of the effect of feeding upon the body-length I cannot do better than cite an observation by Sikora (IX. 1917, p. 276) who measured two females before and after feeding, the result being more striking in the

case of *corporis* because of its habit of gorging (see Nuttall, *Parasitology*, xi. 343–344): Sikora found *capitis* when unfed measured 2.84, fully fed 2.92; *corporis* when starving measured 3.2 by 1.4, fully gorged 3.84 by 1.56 mm.

Finally, owing to the lack of sharply defined structural demarcations, it is difficult to measure the length of the head, thorax and abdomen (especially its segments) more than approximately. Therefore little value necessarily attaches to small differences in size, and seeing that *Pediculus* varies considerably in size, measurements can have but small importance unless applied to a large number of full distended specimens assuming that these are obtainable.

With regard to the width of the abdomen, Fahrenholz states that it is greater in his "*P. capitis marginatus*" than in "*P. capitis angustus*" whose abdomen is elongated, and that the greatest width is attained at the "7th segment" in *assimilis* and at the "5th segment" in *friedenthali*¹.

The value of Fahrenholz's statements bearing on the width of the abdomen is nil, because I have often detected elongated, broad, and intermediate forms of abdomen in both sexes where I have examined large lots of *capitis* and *corporis* that have been collected from single individuals in different parts of the world. Pure strains of *corporis*, raised in the laboratory, have yielded a like result. Moreover, as with the length, the width of the abdomen in both sexes varies according to the state of engorgement, and in females according to the number and size of the ova they contain. Thus in laboratory strains of *corporis* I find that the greatest width ranges forward and backward from the 4th, 4th–5th and 5th abdominal segments (the 5th = Fahrenholz's "7th") in the ♂, whilst in the ♀ the greatest width alternates between the 3rd–4th, 4th, and 5th abdominal segment, the latter most frequently attaining the greatest width (abdominal segment 3 = Fahrenholz's "5th").

For the reasons previously given, no value can be attached to Fahrenholz's statement that the last abdominal segment in "*P. assimilis*" ♀ is equally long and broad, such measurements being fallacious especially in balsam-mounted specimens.

Following the lead of many previous authors, Fahrenholz lays stress on the relative size of *capitis* and *corporis* and of his purported new forms. Examined critically, however, in the light of the subjoined table, these differences should be seen once and for all not to hold good. I have stated this repeatedly in a general way but the appended table will perhaps carry more conviction.

Dismissing the measurements of *capitis* and *corporis* given by older writers, as perhaps wanting in accuracy, I have arranged the following table of measurements by recent authors and newer measurements of my own in accordance

¹ The reader is liable to be confused by Fahrenholz's enumeration of the segments. He does not state that he includes segments 1–3 in the thorax. What appears to him and most observers to be the 1st abdominal segment is numbered 4 by Fahrenholz, but it actually consists of two fused segments (abdominal segments 1 and 2) which bear the first abdominal spiracle. Fahrenholz when he refers to segments 5–6–7 must therefore, in the light of our present knowledge, be understood to refer to abdominal segments 3–4–5 which bear correspondingly numbered abdominal spiracles.

with the size of the males, beginning with the smallest. Where measurements of males are lacking, those of the females are ranged in what appears to be a suitable place. In some of Fahrenholz's measurements I have omitted the second decimal figure. I omit so-called "average" measurements given by different authors, recording only those showing the range in size, smallest to largest, observed in both sexes. Apart from my hitherto unpublished figures, those of Sikora bearing on *capitis* are the only ones regarding which a statement is made as to the number of individuals measured.

Table.

Giving the body-length of the smallest and largest specimens of different forms of *Pediculus humanus* measured by various observers and the author. My measurements were made on well-preserved alcohol specimens.

Length of ♂ in mm.	Length of ♀ in mm.	Name	Number examined	Author and remarks
2.0-2.7	2.4-3.4	<i>capitis</i>	104 ♂ 123 ♀	Sikora, ix. 1917
2.1-2.3	2.3-2.8	" <i>capitis maculatus</i> "	?	Fahrenholz, x. 1916, "shorter than <i>angustus</i> "
2.1-2.6	2.2-3.3	<i>capitis</i>	85 ♂ 81 ♀	Nuttall, Lot 208, woman, Cambridge
2.1-2.8	2.2-3.7	<i>corporis</i>	27 ♂ 77 ♀	Nuttall, Lot 204, woman, Lambeth
2.1-2.8	2.4-2.9	<i>corporis</i>	137 ♂ 94 ♀	Nuttall, Lot 265, Lab. Stock
—	2.19-2.85	" <i>friedenthali</i> "*	?	Fahrenholz, x. 1916. Host: <i>Hylobates</i>
2.19-2.39	2.67-3.06	" <i>capitis angustus</i> "	?	Fahrenholz, x. 1916, "scarcely as large as European <i>capitis</i> "
2.2-2.3	—	<i>capitis</i> *	3 ♂	Nuttall. Hosts: <i>Ateles ater</i> and <i>geoffroyi</i>
—	2.4-2.66	" <i>assimilis</i> "*	?	Fahrenholz, x. 1916. Host: <i>Hylobates</i>
2.2-2.5	2.4-3.3	<i>capitis</i> *	7 ♂ 11 ♀	Nuttall, Lot 315. Host: <i>Ateles paniscus</i>
2.25-3.0	2.75-4	<i>capitis</i>	?	Popoff, 1916
2.35-2.9	2.6-3.3	<i>corporis</i>	31 ♂ 100 ♀	Nuttall, Lot 314, tramp, Cambridge
2.43-2.56	2.61-3.1	* <i>capitis</i>	?	Fahrenholz, 1912
2.5-2.9	3-3.3	" <i>humanus marginatus</i> "	?	Fahrenholz, x. 1916
2.55-3.3	2.85-3.7	<i>corporis</i>	56 ♂ 52 ♀	Nuttall, Lot 212, London
2.7-3.55	2.7-4.2	<i>corporis</i>	220 ♂ 226 ♀	Nuttall, Lot 252, tramp, Cambridge
2.74-3.6	2.74-4.4	<i>corporis</i>	?	Sikora, ix. 1917
2.75-3.75	3-4.75	<i>corporis</i>	?	Popoff, 1916
—	3.29-3.36	" <i>schäffi</i> "*	?	Fahrenholz, 1912. Host: <i>Simia troglodytes</i>
2.9-3.3	3.9-4.37	" <i>humanus chinensis</i> "	?	Fahrenholz, x. 1916, "distinctly larger than <i>marginatus</i> , the largest larger than European louse" (<i>vide infra</i>)
3.02-3.23	3.55-4.20	<i>corporis</i>	?	Fahrenholz, 1912 (European no doubt)
3.75	4-4.5	<i>capitis</i> †	2 ♂ 2 ♀	Popoff, 1916, from Java

* Denotes forms found on apes and monkeys. I have measured two "*schäffi*" ♀♀, both 3.3 mm. in length.* (Fahrenholz, 1910, gives 2.7 as the length, I assume his later statement to be correct.)

† If correctly determined, the size is remarkable. Thus far, in a very large material, I have seen no *capitis* approaching these in size.

The accompanying Chart II, moreover, records measurements made by me on 100 ♀♀ *P. humanus* race *corporis* (Lot 252, from tramp, Cambridge, 1918). The measurements relate to the antennae, head, thorax and abdomen as indicated on the chart, all being arranged along a straight line representing the anterior margin of the thorax. The lice were graded in accordance with the size of the thorax, the largest on the left leading to the smallest to the right. The length of the thorax varied between 1.1 and 0.7 mm. which may be taken as a good index of the relative variation in size in the lot. The great inequality in the length of the abdomen is due to its being more or less filled with blood

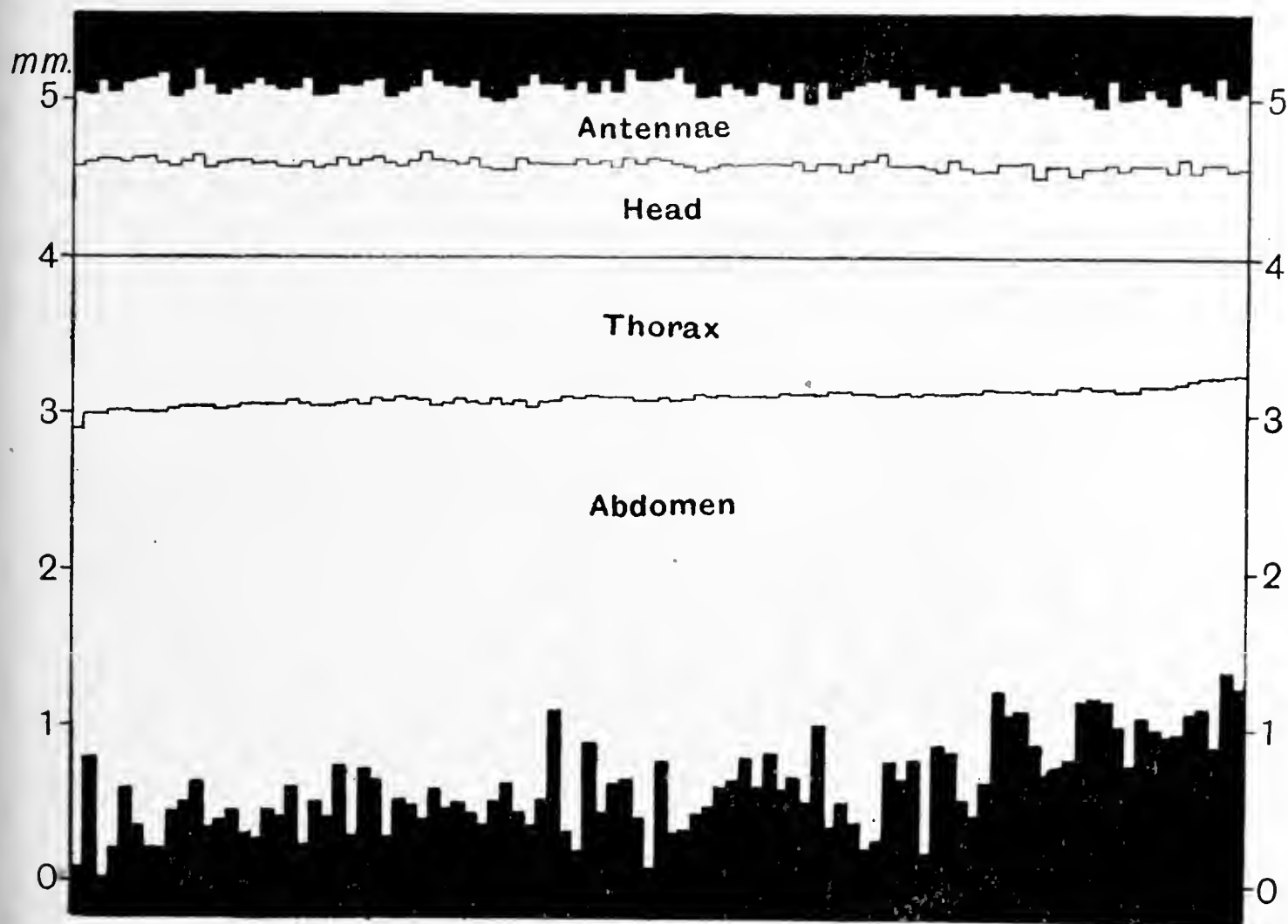


Chart I. Length-measurements of *Pediculus humanus* race *corporis*, 100 ♀♀, arranged in accordance with the length of the thorax.

and ova. The variation in the length of the head and antennae is relatively slight though distinct.

(b) Morphology of *Pediculus*.

The morphological characters and their bearing on Fahrenholz's diagnoses will be considered in sequence, beginning with the head and ending with the legs:

HEAD. There is no doubt that in *capitis* the head is shorter than in *corporis* when *typical* forms are considered. The shortness in *capitis* is due to the more blunted shape of the clypeus which terminates the head anteriorly, and the less elongate form of the neck-like posterior portion. Nevertheless, in pre-

sumably pure breeds of *capitis* and *corporis* respectively, the form of the head varies so that its taxonomic value is largely vitiated. In cross breeds a complete series of intermediate forms may be observed.

ANTENNAE. The statement that *capitis* has shorter antennae than *corporis* likewise holds for typical forms. In presumably pure breeds, a distinct variability is however noticeable, and in cross breeds all kinds of intermediate forms of antennae are seen. In pediculi derived from apes and monkeys, the antennae, like the head, are of the *capitis* type.

In body-lice from negroes ("*P. nigritarum* Fabricius") Fahrenholz describes the antenna as inverted-pear-shaped, the author apparently meaning to refer to the form of the segments composing the antenna, but his wording implies that the whole antenna has this form, this being absurd. He only describes the form from a single ♀ which may well have been a *capitis* with collapsed antennae.

THORAX. It is difficult to define the shape of the thorax dorsally because the outer margin is virtually colourless and the pigmented portions which lie within the margin mislead the eye into accepting them as indicating the form of the thorax. Its shape varies slightly in individual specimens, the lateral contours being more or less convex.

Therefore, when Fahrenholz describes the shape of the thorax in "*P. capitis angustus*" as "parallel sided," in dealing especially with balsam-mounted specimens, the supposed character must be dismissed as valueless. The six large hairs situate dorsally on the thorax in all pediculi I have examined, are stated to be absent in "*P. lobatus*" but I firmly believe that they were merely overlooked by being viewed through balsam (see further under Pigmentation).

ABDOMEN. Fahrenholz, writing of the ♂ abdominal structures, refers to what we have termed the dorsal bands, genital plate, and anterior ventral band. His statements regarding these structures are largely controverted in the following section on Pigmentation, *q.v.*

The Genital plate, in the ♂, is stated by Fahrenholz to be bipartite in "*P. lobatus*" and "*P. capitis maculatus*," and absent in other pediculi. We have, however, shown that this structure varies in normal individuals of one lot (see Keilin and Nuttall, *Parasitology*, XI. 282, fig. 3) and consequently there is no significance to be attached to its consisting of one or two parts, whilst it is never absent.

In the ♀, the *posterior abdominal lobes*, when retracted, become approximated, giving the semblance of a slit in contrast to the usual bilobed appearance of the end of the abdomen when the lobes are protruded. This accounts for what Fahrenholz gives as a specific character in "*P. lobatus*."

The *gonopods*, in the ♀, are stated by Fahrenholz to point back in "*P. friedenthali*," inward in "*P. assimilis*," and to be "short" in both forms, being intermediate in shape between those of *capitis* and *corporis*. I find, however, as already stated elsewhere (Nuttall, *Parasitology*, XI. p. 341), that the form of the gonopods is inconstant in both of these races of *P. humanus* and that

the two forms cannot be distinguished by their gonopods. As to the gonopods pointing "backward" or "inward," this is frequently due to pressure or shrinkage, such abnormal orientation being often seen in balsam mounts.

In both sexes, the *pleurae* vary greatly in appearance according to the degree of chitinization and pigmentation. There is no constant difference between *capitis* and *corporis* in this respect, but in "*P. schäffi*," the 6th abdominal segment certainly projects in an exceptional manner, forming a ridge dorsally and ventrally which is especially evident in ungorged specimens. The 6th abdominal segment does not protrude exceptionally in the adult lice I have examined from monkeys, but it protrudes distinctly in 2nd and 3rd-stage larvae. In corresponding larval stages of human *capitis* it is exceptional to find the 6th abdominal segment protruding somewhat more than the others laterally.

CLAWS. These are described by Fahrenholz as longer or shorter in lice from different races of man, as toothed in "*P. capitis angustus*" and "*P. corporis marginatus*," but not toothed in European lice. These statements are untrue, for the claws vary in length normally and I have often seen toothed claws in European head and body-lice. See also the quotation from Piaget (p. 143).

(c) Pigmentation in *Pediculus*.

The worst blunders committed by Fahrenholz are due to his relying upon pigmentation in his diagnoses, this leading to his referring to various structures as "absent" in some pediculi and "present" in others. Thus

The HEAD in "*P. lobatus*" has dark brown "plates" at the sides.

THORAX. Here the *sternal plate* is said to be present in "*P. corporis nigritarum*" and "*P. capitis angustus*" and "absent" in "*P. lobatus*" and "*P. friedenthali*." In "*P. capitis maculatus*" there occur "holes" in the sternum where hairs arise anteriorly, there being four holes in the ♂ and two in the ♀ sternum.

ABDOMEN. The degree of pigmentation of the *pleurae* serves to distinguish "*P. capitis maculatus*" and "*P. capitis angustus*."

In the ♂, the *dorsal bands* are present in "*P. corporis marginatus*" and "absent" in European body-lice. The *genital plate* is present in "*P. capitis angustus*," "*P. capitis maculatus*" and "*P. humanus chinensis*," but "absent" in European lice. The *ventral band* is a feature in "*P. capitis maculatus*."

In the ♀, on the last abdominal segment, there occur two brown spots dorsally in "*P. capitis maculatus*," indistinct plates (dorsally?) in "*P. capitis angustus*," and two brown plates in "*P. corporis nigritarum*" (based on one ♀!) and negro head-lice.

It is surprising that Fahrenholz should have been so grossly misled. He might at least have taken a warning from Neumann's paper (see quotation on p. 143). Today we know that by raising unpigmented strains of European *P. humanus* on a black background, the "absent" structures of Fahrenholz

are rendered visible even in balsam-mounted specimens! Fahrenholz merely overlooked existing structures because they were unpigmented. Conversely, by raising pigmented lice on white backgrounds, all of Fahrenholz's specific characters may be made to disappear.

Nearly all of the before-mentioned structures in *Pediculus* are illustrated in *Parasitology*, XI. p. 220, Pl. X, figs. 1 and 2 (pigmented and unpigmented specimens raised on black and white respectively), pp. 281 *et seq.*, figs. 2, 3. Others will be considered shortly in papers dealing with our studies on the anatomy of the insect.

It is obviously fatuous for Fahrenholz to describe colour differences in balsam-mounted specimens of lice derived from various races of man ("*P. capitis angustus*" and "*P. capitis maculatus*").

How much he was misled by preconceived notions regarding the different appearances observable in *P. humanus*, is further exemplified by the circumstance that when he found typical *P. humanus* race *corporis* in the Hamburg Museum collections bearing a label stating that they came from negroes, he considered their origin as "very doubtful." He would not believe that negro clothes-lice could be similar to those of Europeans, hence there must have been an error in the labelling (see p. 140). I have many hundreds of specimens which prove that body-lice from negroes and whites are identical in appearance.

Proof that there is no constant relation between the degrees of colouration shown by different parts of the exoskeleton in pigmented P. humanus.

The following observations were made by me with a view to determine if in a series of more or less pigmented specimens the colouration of the various parts follows any uniform law.

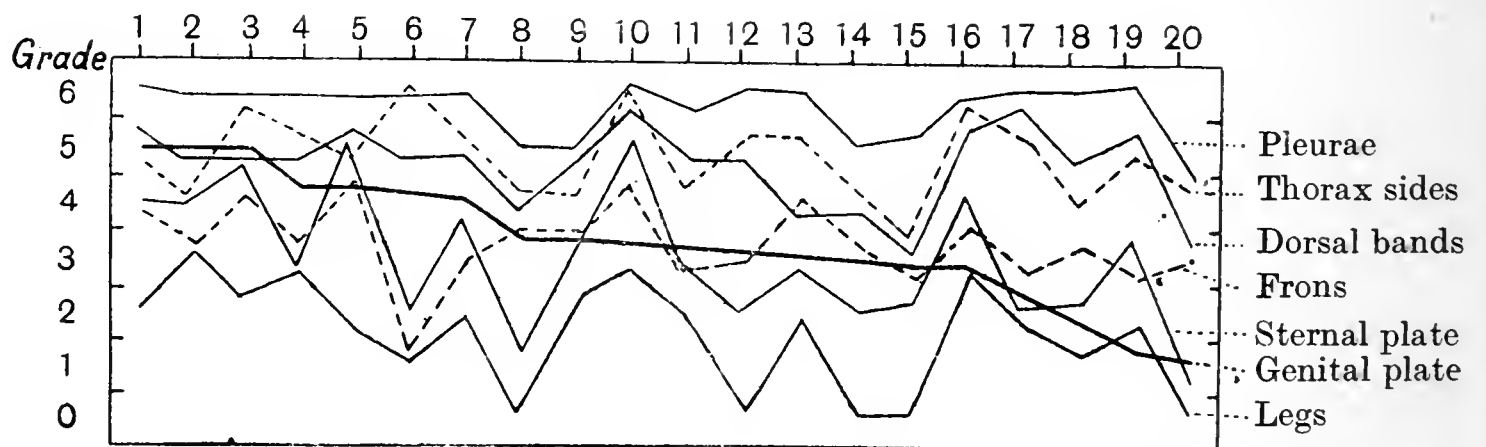


Chart II. Recording the variation in pigmentation shown by 20 selected *Pediculus humanus* race *capitis*, ♂♂, arranged in accordance with the degree of pigmentation shown by the genital plate which is darkest (*i.e.* Grade 5) in specimen 1 and palest in specimen 20, as indicated by the heaviest line among the curves.

From several hundred *capitis* ♂♂ (Lot 252, well-preserved), collected from a negro's head in Tropical Africa, 20 specimens were chosen which showed various degrees of pigmentation when viewed by daylight whilst immersed in alcohol in a dish. A scale giving seven grades of colour from blackish brown

(Grade 6) down to practically colourlessness (Grade 0) was painted on paper with water-colours, and the various degrees of pigmentation exhibited by the lice were recorded on a chart with the aid of the colour scale. The specimens were at first charted in no particular order, but afterwards they were ordered in accordance with the degree of pigmentation shown by their genital plates. The results of this colorimetric study are shown in the accompanying Chart II wherein the thickest line among the curves records the gradual fall in the degree of pigmentation shown by the genital plate in 20 ♂♂. It will be noticed at once that the degree of colouration of the genital plate bears no relation to that of the other structures. This plate may even be colourless whilst all the other structures are distinctly pigmented. The degree of pigmentation appears fairly uniform in the pleurae, whilst in some other structures (*i.e.* frons, sides of thorax, etc.) it often rises and falls, sometimes synchronizing with the varying colouration of other parts and sometimes not. In short, there is often agreement but no constant relation between the degrees of pigmentation shown by the different parts.

These observations bear on my criticism of Fahrenholz's work in so far as they prove that considerable individual variation may occur in the degree of pigmentation shown by different parts of the exoskeleton of *Pediculus* and that this has to be taken into account in all descriptions.

III.

SUPPLEMENTARY NOTE UPON THE SYNONYMY OF *PEDICULUS HUMANUS*.

The following synonymy requires to be added to that supplied in my previous paper in *Parasitology*, XI. pp. 334-7:

(a) *Pediculus humanus* race *corporis*.

- 1805. *Pediculus nigritarum* Fabricius 1805, p. 340. From negroes.
- 1816. *Pediculus albidior* Olfers 1816, p. 81, cited by Fahrenholz, VII. 1916, p. 270.
- 1816. *Pediculus nigrescens* Olfers 1816 (*fide* Fahrenholz, *loc. cit.*).
- 1910. *Pediculus capitis vestimenti* Neumann 1910, p. 412. "Il me paraît logique de conclure qu'il conviendrait de faire descendre *P. vestimenti* du rang d'espèce à celui de sous-espèce et de le considérer comme *P. capitis vestimenti*." This procedure is not permissible under the rules of zoological nomenclature, for *humanus* has priority over *capitis* as a specific name and *corporis* has priority over *vestimenti* as a sub-specific varietal, or racial name.
- 1915. *Pediculus corporis* var. *nigritarum* (Fabricius 1805) Fahrenholz 1915, p. 597, fig. 1.
- 1916. *Pediculus humanus chinensis* Fahrenholz x. 1916, p. 87. From Chinese.

1916. *Pediculus humanus marginatus* (Fahrenholz 1915) Fahrenholz VII. 1916, p. 270. From Japanese.
1916. "*Pediculus corporis nigritarum*" of Fahrenholz VII. 1916, p. 270.
1917. "*Pediculus humanus humanus* L" in Fahrenholz 1917, p. 1 (reprint).

(b) *Pediculus humanus* race *capitis*.

1816. *Pediculus pubescens* Olfers 1816, p. 81, cited by Fahrenholz, VII. 1916, p. 270.
1916. *Pediculus capitis angustus* (Fahrenholz 1915) Fahrenholz x. 1916, p. 88. From Japanese.
1916. *Pediculus capitis maculatus* (Fahrenholz 1915) Fahrenholz VII. 1916, p. 271. From Negroes.
1916. *Pediculus friedenthali* Fahrenholz x. 1916, p. 88. From *Hylobates mülleri*.
1917. "*Pediculus capitis capitis* de Geer" in Fahrenholz 1917, p. 2 (reprint).
1919. *Pediculus assimilis* Fahrenholz, 1919, p. 27 (= *P. oblongus* Fahrenholz renamed).

CONCLUSIONS.

The evidence adduced in this paper points to the imperative necessity for the employment of more scientific method in the description and differentiation of species of Anoplura. It is shown that a leading authority on the group has blundered greatly in dealing with *Pediculus*, and this may be taken as a fair example (compare *Haematopinus*, etc.) of the manner in which many purported species, sub-species and varieties of Siphunculata are being foisted into the literature by that author. Surely it is time to call a halt to this unwarranted process of burdening science with useless specific and other names that are destined in many cases to fall into synonymy.

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ON TWO NEW GREGARINES, *ALLANTOCYSTIS DASYHELEI* N.G., N.SP., AND *DENDRORHYNCHUS SYSTEMI* N.G., N.SP., PARASITIC IN THE ALIMENTARY CANAL OF THE DIPTEROUS LARVAE, *DASYHELEA OBSCURA* WINN. AND *SYSTEMUS* SP.

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(With Plate X and 2 Text-figures.)

1. *ALLANTOCYSTIS DASYHELEI* n.g., n.sp.

THE host of this gregarine is the larva of a Ceratopogonid: *Dasyhelea obscura* Winnertz, which, as I have previously mentioned¹, lives in the decomposed sap filling the infected wounds of the Elm and Horse-chestnut trees in Cambridge (at Newnham and along the Queen's Road). The parasitised larvae were found late in the season, about the end of September, they were all young and never heavily infected, containing from three to eight and exceptionally twelve parasites in different developmental stages. The gregarine seems to be rare as among several hundred of these larvae examined only twelve yielded this interesting parasite.

All the stages of *Allantocystis dasyhelei* occur in the midgut of its host, in the space between the intestinal epithelium and the peritrophic tube, and they always lie with their main axis parallel to that of the larva.

The full-grown sporont is elongated, 65–75 μ long and 20–22 μ across its widest portion (Pl. X, fig. 3). The sporont moves slowly and its body easily bends on meeting an obstacle. The ectoplasmic layer is very thin except anteriorly. The endoplasm is very granular and completely masks the nucleus, which can be detected, however, by compressing or staining the parasite; the nucleus then shows a structure typical of gregarines, *i.e.* a large vesicle with a distinct karyosome. The youngest stages of the parasite observed were the sporonts, 25–35 μ in length, differing from the full-grown forms only in having their endoplasm less granular and in containing several patches of very small yellowish granules (Pl. X, figs. 1 and 2). In sexual association the gregarines attach themselves to one another by their anterior extremities.

¹ See D. Keilin, *Parasitology*, this volume, p. 85.

So far, the characters of this gregarine agree with all we know of the general structure of the group. The peculiar character of the species is confined to the structure of its cyst. It is well known that in gregarines two full-grown sporonts (or gametocytes), when associated for reproduction, contract their body and form a more or less spherical mass which is surrounded by a common cyst.

In the case of *Allantocystis dasyhelei*, on the contrary, the two sporonts associated for reproduction, without changing their form, secrete a very elongated sausage-like cyst¹, measuring 140–150 μ in length and only 20 μ in width (Pl. X, fig. 4).

Once the cyst is formed, the protoplasm slightly retracts, the septum which previously separated the two gametocytes disappears, and the interior of the cyst forms a fused mass consisting of dark granular protoplasm with irregular contours. The gametes and the sporoblasts seem to be formed by the ordinary process common to almost all gregarines. In a few cases I could follow *in vitro* the formation of the sporoblasts; a freshly formed cyst (similar to that represented in Pl. X, fig. 4) left in normal salt solution at 7.15 p.m. one day, was found at 10 o'clock the next morning with completely formed sporoblasts as represented in Pl. X, fig. 5.

The sporocysts (Pl. X, figs. 6 and 7) are spindle-shaped, with one side slightly more prominent than the other; they are 18 μ long and 6.5 μ wide.

It is important to note here that the only gregarine known, where sporonts do not contract their body before sporulation, is the very interesting form found by Léger (1892, pp. 159–160) in the coelom of the Polychaete worm *Glycera*, and described by him under the name *Ceratospora mirabiles* Léger. This gregarine, however, differs from *Allantocystis* and all other known gregarines in a very important character, namely, the associated sporonts remain always separated from each other by a septum, and the gametes of each sporont develop parthenogenetically into spores. It would be of great interest to study cytologically the development of these spores, but unfortunately, as Léger mentioned, this gregarine is rare. The peculiar shape of the cysts of *Allantocystis* makes it difficult to define the systematic position of this genus.

DENDRORHYNCHUS SYSTENI n.g., n.sp.

This gregarine was found in a larva of a Dolichopodid fly: *Systemus* sp., probably *Systemus scholtzii* Loew², which occurs with the larva of *Dasyhelea obscura* Winn. in the decomposed sap of the Elm tree; but, while the latter larva is saprophagous and feeds upon decomposed sap, the larvae of *Systemus*

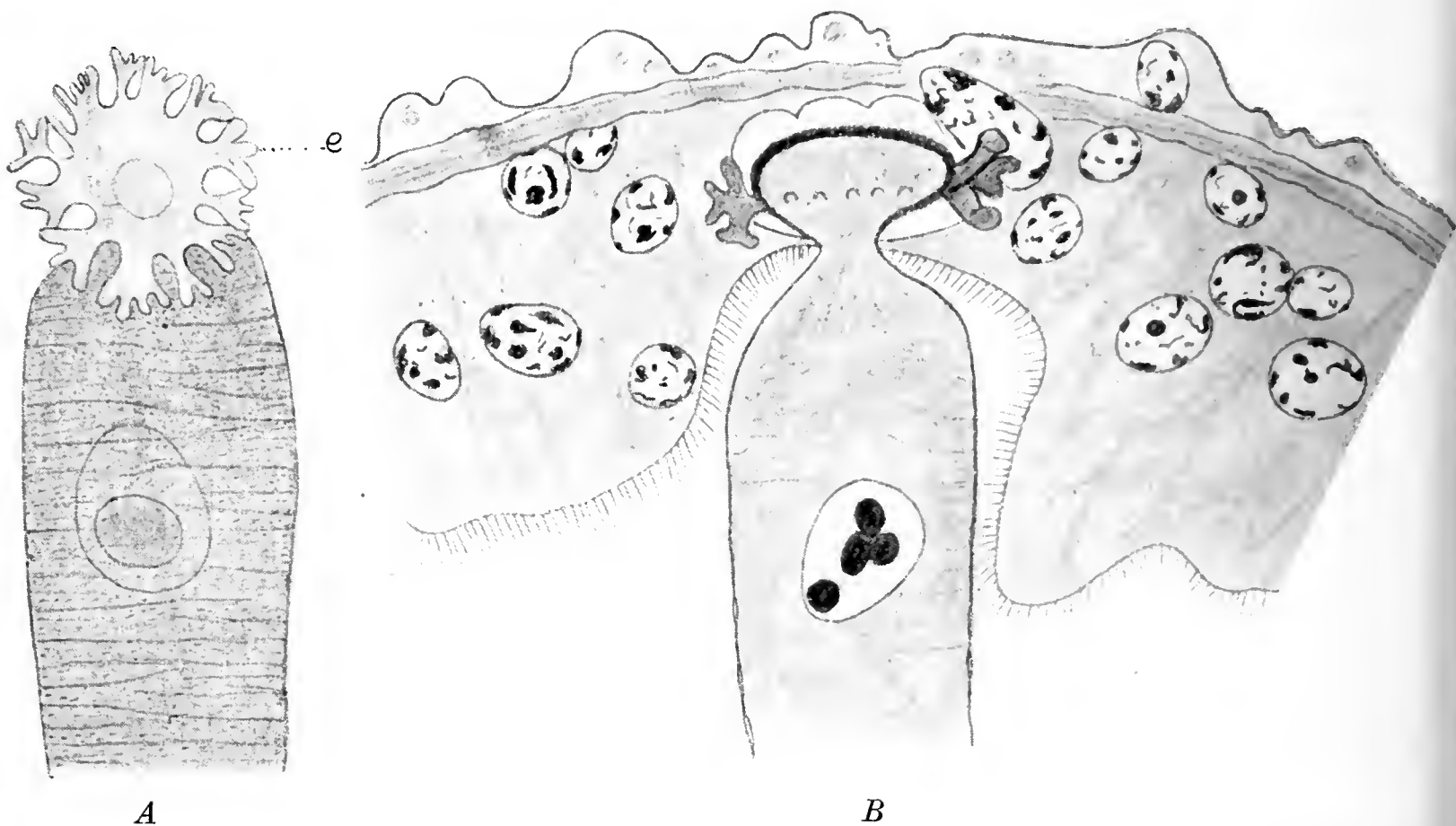
¹ Whence the generic name *Allantocystis*.

² The host was kindly identified by Mr C. G. Lamb as belonging to the genus *Systemus* being probably *S. scholtzii* Loew, but for the exact identification of the species it is important to examine the ♂♂, which unfortunately I failed to obtain. The ♀ of this species is difficult to distinguish from that of *S. adpropinquans* Loew whose larvae were found by Laboulbène (1873) in the sap of the Elm tree.

is carnivorous and lives upon *Dasyhelea* and several other dipterous larvae which are always found associated.

The gregarine, in different stages of its development, occurs only in the midgut of its host, where it can remain for a long time as a trophozoite, attaining, at this stage, the size of a full-grown sporont, *i.e.* being $255\ \mu$ long and $18.5\text{--}20\ \mu$ wide.

In all the stages the gregarine moves very slowly and easily bends and contracts its body (Pl. X, figs. 11 and 12), which looks often as if it were composed of numerous rings; these curved specimens can frequently be seen performing a continual rotating movement. The epimerite of the cephalont

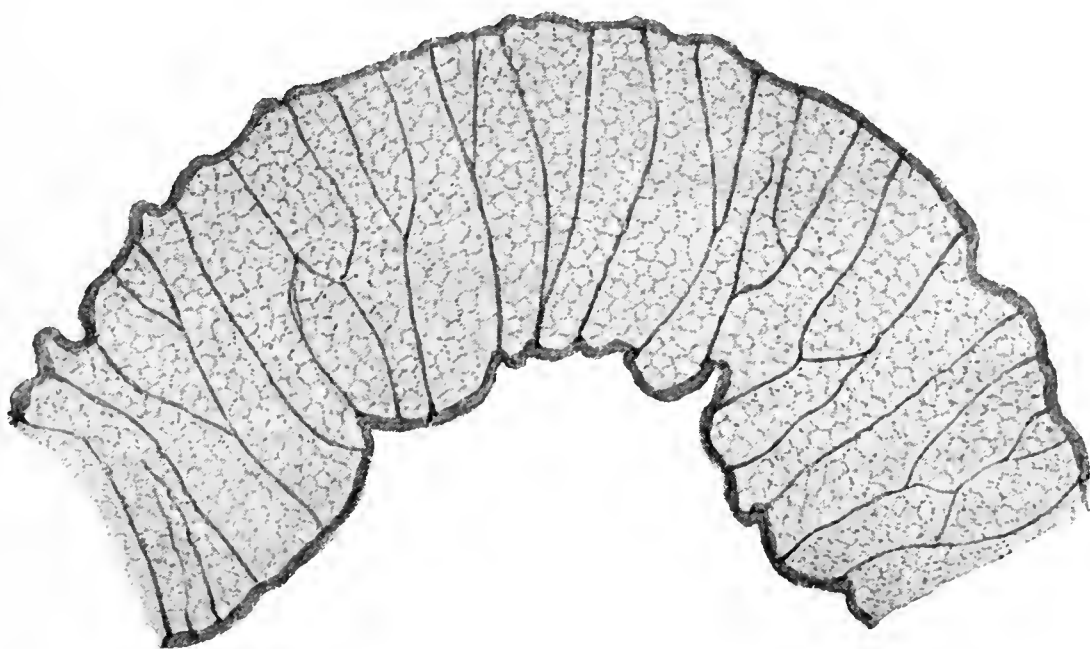


Text-fig. 1. *Dendrorhynchus systemi*: *A*, the anterior portion of a trophozoite showing the epimerite (*e*). *B*, section of a trophozoite with its epimerite fixed in the epithelium of the host's midgut.

(Text-fig. 1, *A* and *B*) has the form of a disc surrounded by numerous more or less ramified papillae.

At various stages, the cephalont, shedding off the epimerite, can separate itself from the host's epithelial cell and become a free moving sporont (Pl. X, figs. 8, 9 and 10). The body of the sporont is elongated with the posterior end slightly curved and of irregular contour; it does not seem to be divided into two segments, pro- and deutomerite, as is usual in cephaline gregarines. The ectoplasm is not very thick and shows well the longitudinally striated epicyte. The living, and especially the fixed and stained specimens, show, under the epicyte, a network of very well defined circular fibrils with oblique anastomoses which surround the whole body of the gregarine (Text-fig. 2). They undoubtedly correspond to the myocyte fibrils of *Gregarina munieri* described by Schneider (1875), although in this case the network is much

denser than that of *Dendrorhynchus*. In some specimens of *Dendrorhynchus* it was rather difficult to ascertain that the transverse fibrils were only superficial and did not penetrate deeper in the endoplasm and form a series of septae similar to those of *Taeniocystis mira* described by Léger (1906). The endoplasm is very granular, the granules being disposed in transverse parallel planes. The vesicular nucleus, with usually several karyosomes, is situated in the anterior portion of the gregarine. I have not yet been able to find the sexually associated forms, though several times I found the subspherical cysts, 60 to 80 μ in diameter, but unfortunately none of these cysts showed the spores. On the other hand in several young *Systemus* larvae, containing the trophozoites of *Dendrorhynchus*, I found the cysts of elongated shape, about 100 μ long, measuring 40 μ across their widest portion and having one side prominent and the other flattened (Pl. X, fig. 13). These cysts, which probably belong to the same gregarine, contained ripe spindle-shaped spores 18–19 μ long and 7 μ wide (Pl. X, figs. 14 and 15).



Text-fig. 2. *Dendrorhynchus systemi*: a portion of a trophozoite fixed and stained, showing myocyte fibrils.

The shape of the epimerite of this gregarine recalls that of *Rhopalonia stella* Léger and *Echinomera hispida* (Schneider) Labbé, and I am inclined to think that the genus *Dendrorhynchus* can be placed near the latter two genera in the family of DACTYLOPHORIDAE Léger (1892).

It is interesting to note that the *Systemus* larva, the host of *Dendrorhynchus*, often contains a Schizogregarine bearing much resemblance to *Schizocystis gregarinoides* Léger (1910), which, with *Taeniocystis mira* Léger (1906), live in the larvae of *Ceratopogon soltitialis*.

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EXPLANATION OF PLATE X.

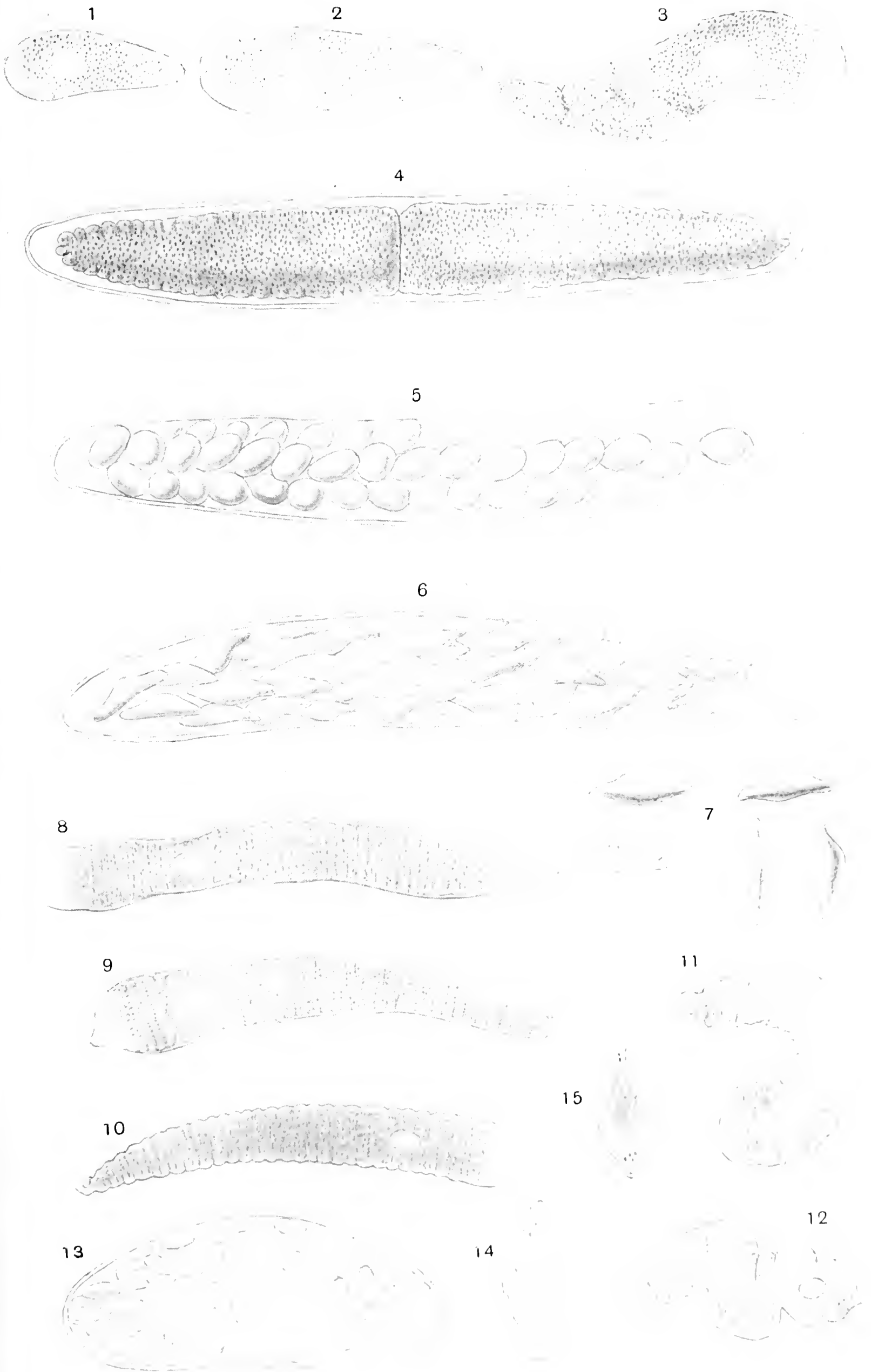
All the figures were drawn from living specimens.

Allantocystis dasyhelei. × 800.

- Figs. 1 and 2. Young sporonts.
- Fig. 3. Full grown sporont.
- Fig. 4. Cyst freshly secreted by a copula; the thickness of the cyst-wall is exaggerated.
- Fig. 5. Cyst with formed sporoblasts.
- Fig. 6. Cyst with spores.
- Fig. 7. Spores seen from different sides.

Dendrorhynchus systemi. Figs. 8-12. × 490.

- Figs. 8, 9 and 10. Different stages of sporonts.
- Figs. 11 and 12. Contracted sporonts.
- Fig. 13. Cyst with spores, probably belonging to *D. systemi*. × 490.
- Figs. 14 and 15. Spores of the same cyst. × 1000.



SPERMATOGENESIS IN *IXODES RICINUS* LINN.

BY ERIK NORDENSKIÖLD, STOCKHOLM.

(With Plate XI.)

It has long been known that ticks possess spermatozoa that differ considerably from the typical flagellate form. In similar cases science has always attempted to trace the aberrant type back to the normal with a view to identifying its constituent parts. Many forms, even those which at first sight appear incapable of being compared with the normal, have recently been thoroughly analysed in this manner. On the other hand there occur several types of spermium whose parts it is impossible to compare with those of the normal type and in these instances all that can be done is to identify the elements that take part in the fertilization, viz. the nucleus and centrosome.

Very little has previously been published on spermatogenesis in ticks. A. Bonnet (1907) refers to it and Samson (1909) has published a preliminary note on the subject whilst Robinson and Davidson (1914) refer but briefly thereto. None of these publications fulfils the demands of a modern investigation of spermatogenesis; neither as regards terminology, nor in the attempt to trace the development of the different elements is any attention paid to former scientific results on this subject. Under these circumstances it is scarcely possible to discuss the details of these papers; some of the stages described in them can by the aid of the figures be put into their proper places, others are impossible to identify.

In an earlier paper (1909), I described the results of some of my researches on spermatogenesis in *Ixodes ricinus*¹. The present paper contains several corrections relating to statements made in my previous communication, these corrections being based upon more detailed investigations that have thrown new light on several matters. The earlier results were obtained chiefly from material fixed in Carnoy's alcohol-chloroform-acetic acid. Meanwhile it has been proved that this method, excellent as it is for several other purposes, is very unsuitable for studies on spermatogenesis. The results described in the present paper were obtained mainly from material fixed in Flemming's chromosmic-acetic acid, due attention always being paid also to the Carnoy-material.

Since my earlier paper appeared, our knowledge of spermatogenesis and its accompanying phenomena has considerably increased. Several matters,

¹ Nordenskiöld (1909). Zur Spermatogenese von *Ixodes reduvius*. *Zoologischer Anzeiger*, xxxiv. 511-516, 10 figs. (*Ixodes reduvius* Latreille, 1844 = *Ixodes ricinus* Linn. 1758.)

i.e. the conjugation and the reduction of the chromosomes, have become special subjects for investigation and such researches are becoming increasingly specialized, because they demand specially adapted material and a great sacrifice of time.

Owing to lack of opportunity for the complete elucidation of the various problems concerned in spermatogenesis, and the *Ixodes* material not being very suitable for the purposes of investigation, I shall deal summarily with certain aspects of the subject and confine myself here to tracing the changes that are undergone by the young spermatid whilst developing to the full-grown spermatozoon.

The spermatogonium. Among the elements of the testis the spermatogonium is, as usual, the smallest (Pl. XI, fig. 1); its plasma is homogeneous, finely granular, without any distinct inclusions; its nucleus is relatively large, spherical, with a large nucleolus, a fine and close linin-network, and, scattered over this, a very finely granular chromatin. At the mitosis, this substance forms 28 short, slightly curved, rod-shaped chromosomes. The centrosome appears at the division as a very conspicuous, triangular corpuscle; but as will be shown, it is hard to follow its later development. The spermatogonial divisions appear in the earlier part of the nymphal stage at a time when the whole gonad still forms a narrow tube with homogeneous contents. The later development of the gonad cells appears, on the other hand, only at the beginning of the last moult before the prosopon stage.

The spermatocyte. The resting spermatocyte of the first order (Pl. XI, fig. 2) shows a round or, when pressed by crowding, a somewhat angular shape. The globular nucleus forms the largest part of the cell. It shows a well-developed linin-network, on which the chromatin is scattered in the form of slender threads. The nucleolus is large and the nuclear membrane thin. The surrounding plasma has a double structure: the inner zone around the nucleus is uniformly fine-granular and contains larger or smaller aggregations of mitochondria. Around this inner zone is seen an outer layer, somewhat resembling a zona pellucida, and composed, as a closer investigation will show, of a denser and more deeply staining matrix, through which extends a system of diffuse less deeply staining processes from the inner plasma. The resting spermatocyte of the second order differs from that of the first order only by its smaller size.

The spermatid is, as usual, smaller than the spermatocytes. Beginning with the telophase of the second maturation division (Pl. XI, fig. 3) there is seen, along with the redissolving chromosomes, the centrosome closely attached to the nucleus, often hidden among the chromosomes, and always, as a matter of fact, opposite the spindle. The spermatid, when full grown (Pl. XI, fig. 4), shows a nucleus with generally the same arrangement of chromatin and achromatin as in the spermatocytes, for it shows a reticular linin and, scattered in it, a granular chromatin; the nucleolus is conspicuous by its size, and is indeed far more voluminous than in the former cells. The

plasma has also the same arrangement of its chief components: the outer layer has the same fibrillar structure as previously described in the spermatocytes; it is however much thicker in comparison with the inner layer; the latter also shows the same granular substance and the same aggregations of mitochondria around the nucleus.

The further development of the spermatid begins, as usual, with a change in the position of the nucleus which moves through the plasma to the periphery and becomes closely attached to the cell membrane (Pl. XI, fig. 5). Here it must be observed that the final position of the nucleus depends on that of the spindle of the last division: the nucleus always passes to the side where the remains of the spindle appear as an appendix to the plasma (Pl. XI, fig. 6). This circumstance makes it possible to follow the different constituents of the spermatid during its subsequent intricate process of metamorphosis.

The above-mentioned change of place is the signal for a series of modifications in the shape of the nucleus. At first its constitution is altered: its several components, linin, chromatin and nucleolus seem to be effaced and to emerge as a homogeneous, uniformly colourable matter. This substance shrinks and becomes concentrated towards the inner part of the nuclear vesicle (Pl. XI, fig. 5), the outer part being occupied only by nuclear fluid; later on the nuclear membrane shrinks round the substance, thus considerably diminishing the volume of the nucleus. There now begins a still more important change in the shape of the nucleus: first it becomes somewhat pear-shaped, later on it elongates and becomes rod-shaped (Pl. XI, figs. 6 to 12). In transverse sections this nuclear rod (Pl. XI, fig. 9) shows a peculiar structure, owing to the disposal of the chromatin in peripheral longitudinal filaments surrounding a central chromatin cord. In order to define the orientation of the rod, the exterior end, which is turned towards the fragment of the spindle, will be termed hereafter the external end, and the opposite end will be called the internal end.

During this nuclear process important changes are taking place in the plasma: (1) *the endoplasma*. Here the mitochondria, which, as is shown above, were crowded around the nucleus in the form of several well-circumscribed masses, are dispersed homogeneously through the whole endoplasma (Pl. XI, figs. 4, 5), and at this stage it is impossible to discern them as a special ingredient of this matter. Through this dissolution of the mitochondria the endoplasma itself acquires a greater affinity to stains, which characterizes it during the following stages. Round the external end of the nucleus the endoplasma penetrates through the ectoplasma and here appears at the surface of the cell (Pl. XI, figs. 8, 10, 11). At the opposite side it also expands and forms a very characteristic process, at first obtusely conical (Fig. 8), which later on comes to play a very important part in the development. Even more remarkable are the changes in (2) *the ectoplasma*. The original fibrillar structure of the plasma-zone disappears; the fibrils melt away and simultaneously the whole layer increases considerably in thickness. In the now nearly homogeneous layer, appear several large vesicles, distinguishable

from the surrounding plasma by their clearer contents and increasing greatly in size (Pl. XI, figs. 11, 13, 14). Among these vesicles one especially, situated opposite the internal end of the nucleus, attains a considerable size (Fig. 15). Finally the vesicles run together and occupy the whole mass of the ectoplasma; they are, as this shows, also plasma structures and not liquid inclusions.

The part played by the centrosome during this evolution of nucleus and plasma is not yet described. When last seen, it was at the telophase of the second maturation division, half hidden among the chromosomes at the part of the cell opposite the spindle. The most difficult part of the whole investigation was the subsequent following of the centrosome. The very important part it plays everywhere in the development of the spermatozoon makes an accurate observation of it through the several phases of the cell-metamorphosis indispensable. One meets indeed not infrequently with investigations on spermatogenesis wherein the centrosome has been invisible at some stage, and in which its behaviour is only traceable by inference. Such is also unfortunately the case with our subject. In the meta- and ana-phases of the mitoses the centrosomes are easy to observe as triangular points, surrounded by a strong radiation, but, already at the telophase, the centrosome, as has been explained, is not always easy to find. And in the young spermatids it is generally very difficult to distinguish the centrosome from the similarly granular mitochondria: none of the special staining methods recommended in the literature for this purpose was of any use here, although many have been tried; the mitochondria cannot be decolourized in such a way that the centrosome remains, nor is this element, as is very often the case in other objects, surrounded by any characteristic plasmatic structure such as radiation or clear plasma zone, which could be used as a means of identification. On close examination it is indeed possible to fix the position of the centrosome at least approximately. Here the telophase must be the starting point. In it the centrosome lies, as has been already mentioned, close to the chromosomes and always at the pole of the nucleus opposite to the mitotic spindle. At this pole there is, even after the reconstruction of the spermatid nucleus, in favourable preparations, a minute deeply coloured structure, which must undoubtedly be identified with the centrosome (Fig. 3). As the nucleus begins to approach the spindle side of the cell surface, and later on becomes rod-shaped, it is to be expected, that the centrosome would even still be found at the internal end of the nucleus. As a matter of fact, at this place, in many preparations, can be seen a very small, deeply staining granule (Fig. 7), which may be regarded as the centrosome. In the following stages the centrosome unites even more closely with the internal end of the rod-like nucleus (Figs. 10-12, 14). It appears in close union with the end of the nucleus, surrounded by a clear zone, and opposite the previously mentioned conical projection of the endoplasma.

In the following development the nucleus, the centrosome and the mitochondria of the endoplasma play the most considerable part and consequently

these three parts of the spermatid must be specially studied. This development is characterized by a still more considerable elongation of the nuclear rod. The internal end of it begins to bend in various directions (Figs. 12–16). The external end of the nucleus also assumes a characteristic shape, its point becoming bluntly elongated, almost club-shaped (Figs. 15–18). This end retains its position at the surface of the nucleus, and forms the fixed point, round which the other parts of the nuclear rod move; the latter is sometimes **S**-shaped, sometimes spiral-shaped. As development proceeds the rod becomes progressively thinner and more convoluted.

Parallel to this development of the nucleus there are equally important changes in the plasma. The endoplasma, as we left it, when it had reached the cell membrane in the neighbourhood of the external end of the nucleus, extends its surface from this point and grows in this way all around the ectoplasma (Figs. 10, 12, 16–18). And at the same time the above-described endoplasmatic projection, opposite the internal end of the nucleus, penetrates deeper into the ectoplasma and assumes a more pointed shape (Figs. 14, 15, 17). In the protuberance there accumulates a considerable quantity of mitochondria from the endoplasma, the whole projection acquiring a deep colour (Fig. 17). With this mitochondrial body the internal end of the nucleus enters into a relation, thus making it most difficult to observe the development of the centrosome in the phase that now follows.

As the internal end of the nucleus begins its movements, the centrosome follows them. It is often seen in the first stages of the bent nucleus at the concave side of the rod, the concavity of which seems to be occupied by the clear zone round the centrosome (Figs. 11, 12). Later on the centrosome seems to become attached to the end of the nucleus and surrounded by a now very diminished clear zone (Fig. 14). Thus situated, it follows for a while the movements of the nucleus as shown by Fig. 16. But after this it is soon lost to sight. At the same time we see that the end of the nucleus extends into the mitochondrial body of the endoplasma (Figs. 15, 17) and therefore we may conclude that at this phase the centrosome leaves the nucleus and remains among the mitochondria. It is not possible to adduce any strict proof of this statement, since, as is mentioned above, it is impossible to decolourize the mitochondria in a way that leaves the centrosome stained. Nevertheless this supposition is very probable. The loosening of the connection between nucleus and centrosome happens at the same time as the nucleus and the mitochondria come into contact, moreover, the mitochondrial body in the succeeding development plays a part scarcely intelligible without assuming union with the centrosome. This being the case it will be assumed in the following that the above statement is correct.

The next stage of the spermatogenesis effects an ejection of a part of the plasma such as very often follows the transformation of the spermatid to spermatozoon. It begins with a considerable expansion of the plasma-vesicle (Pl. XI, figs. 17–19), leading to the formation of a large sac of ectoplasm from

the wall of which the endoplasm, with the contents of the more or less coiled nucleus, centrosome-body and mitochondria, protrudes as a cap-shaped boss. The plasma ejection begins with the formation of a protuberance from the endoplasm (Pl. XI, fig. 20 *a*), at first conical, later increasing considerably (Figs. 20 *b* and *c*); lastly it separates itself from the same plasma body and forms a tail-shaped appendix fastened to the external end of the nucleus (Figs. 20 *b*, 21–23). This end of the nucleus pushes itself entirely outside the cell, the curved internal end of the nucleus alone remaining in the plasma and in this way keeping up the connection with the original plasma and the tail-shaped part of it. This tail has originally a purely plasmatic character, being formed of an internal, granular, mitochondrial cord, covered by a quite thin hyaline plasma-layer (Figs. 21, 22)¹. After a while this conformation alters: it now forms a hollow tube through which several deeply-staining filaments pass, and is attached to the now funnel-like end of the nucleus (Figs. 23, 24). From the bottom of the funnel a thin cord is stretched along the whole appendix. Lastly the whole tail shrinks and assumes the form of a knotted thread, which finally vanishes entirely (Fig. 24). This process thus forms a plasma ejection of the sort that has been observed very commonly in the spermatogenic development.

During this development of the nuclear appendix the cell plasma undergoes equally remarkable changes. The ectoplasma-vesicle, rounded at the beginning (Figs. 18, 19), grows in length, at first assuming a club-like and later a rod-like shape (Figs. 21, 22), and at the same time the centrosomal corpuscle—this name seems suitable for the centrosome with surrounding mitochondria—passes from the endoplasm to the ectoplasma, where it takes its position near the boundary between the two (Fig. 24). The endoplasm, which was formerly gathered together in a solid mass, spreads in an ever thinner layer over the rounded front end of the ectoplasma-rod leaving the centrosomal corpuscle at the point of the rod (Pl. XI, fig. 24).

The movements of the nucleus continue during these changes in the plasma: as the endoplasm still has its compact form, the nucleus is seen situated either at right angles to the plasma-rod or as a continuation of it (Figs. 22, 23). Later on, when the endoplasm has spread, the nucleus is situated on the surface of the plasma-rod (Figs. 24, 25, *a*, *b*) and begins finally to roll itself up at its anterior end (Fig. 26), but stretches out again along the side of the plasma-rod. At the same time the centrosomal corpuscle becomes conical in shape and assumes a position at the end of the plasma-rod in close contact with the end of the nucleus, as shown in Fig. 31, and in transverse section in Fig. 27.

Behind this centrosomal cone there now appears along the centre of the plasma-rod a stainable central cord (Figs. 26, 30, 31), the origin and signifi-

¹ This can be specially shown by material fixed with Flemming's liquid. On Carnoy-material the tail shows from the beginning a sort of skeleton-structure. Unfortunately I have got no commencing stage of the tail-formation in Flemming-material.

cance of which are two of the most difficult problems in the whole investigation. The fact is that at the stage in question there appear in the plasma-rod longitudinal folds, which are more colourable than the plasma itself, and as it seems, at least in some transverse sections, are formed by invagination of the surface (Figs. 28, 29), from which it seems possible to conclude, that the cord mentioned above is formed in this way, by folding and separation of the fold from the surface. The spermia, which are always curved and folded, here prevent to a very great extent the observation of the process, but cross-sections, as mentioned above, make this interpretation of the conditions at least possible.

Through the definitive location of the nucleus along the plasma-rod and the appearance of the above-mentioned cord the spermatozoon acquires the shape shown in the vas deferens of the male (Figs. 30, 31). At this stage we see the rod-like, laterally placed nucleus, the conical centrosomal corpuscle, united with the nucleus, the plasma-rod with its staining cord. The spermatozoon of the tick is, as shown by this, atypical, and, like that of several crustaceans, cannot be compared with regard to its several components, with the common spermatozoon type. A desire to attain this object led me astray in my preliminary investigation, but the errors in my previous publication can easily be rectified by the results published here.

The work, of which the results are herein published, was begun at the Zoological Laboratory at Marburg and finished after a long interval of time at the Zoological Institute at Christiania. To Professors Korschelt and Tönniges at Marburg and Bonnevie at Christiania I am much indebted for the helpful advice I have received during my work, for which I here beg to offer them my most respectful thanks.

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Note.—From the extensive literature on spermatogenesis may here be quoted only some authors that have been especially consulted in connection with the work, the results of which are here described; a great many others, that have only occasionally been consulted, may from considerations of space be omitted.

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EXPLANATION OF PLATE XI.

The figures have been drawn with an apochromatic Zeiss lens 2 mm., and a compensation ocular No. 8 (tube-length 160 mm.), giving a magnification of 1000 diameters; they were projected on the working table through a camera of Abbe's model. Figs. 1-5, 20, 23 and 27 were drawn from preparations fixed in Carnoy, the others from Flemming preparations. Heidenhain's iron-alum haematoxylin was used as a stain throughout.

- Fig. 1. Spermatogonium in resting-phase.
 Fig. 2. Resting spermatocyte of first order.
 Fig. 3. Telophase of second maturation division, with centrosome and remnant of spindle.

THE SPERMATID.

- Fig. 4. Resting phase.
 Fig. 5. Showing contraction of nucleus.
 Fig. 6. The same, with remnant of spindle visible.
 Fig. 7. Elongating nucleus, centrosome, and thickening of ectoplasm.
 Fig. 8. The nucleus has assumed a rod-shaped form, vesicles are appearing in the ectoplasm and the endoplasm shows aggregations of mitochondria, and conical protrusion.
 Fig. 9. Nucleus seen in transverse section.
 Figs. 10-11. Successive developments of nucleus; centrosome visible.
 Fig. 12. The same, showing endoplasm growing round ectoplasm, and appearance of metachondrial bodies.
 Figs. 13-15. Showing convolution of the nucleus, enlargement of the ectoplasm-vesicles, and further development of the mitochondrial body.
 Fig. 16. Showing growth of endoplasm round ectoplasm.
 Fig. 17. The same, the centrosome at extremity of nucleus.
 Figs. 18-19. Showing expansion of the ectoplasm-vesicle, and the protuberant mass of endoplasm containing the coiled nucleus and mitochondrial body.

DEVELOPMENT OF THE SPERMATOZOON.

- Fig. 20 *a, b, c*. Phase of plasma expulsion and formation of tail-shaped appendix.
 Figs. 21-22. Showing the plasma appendix separating from the cell body, elongation of ectoplasm-vesicle, and nuclear changes.
 Fig. 23. The plasma appendix begins to disappear.
 Fig. 24. The endoplasm forms a thin covering to the ectoplasm, with mitochondria between the two.
 Fig. 25 *a, b*. Successive movements of the nucleus; the mitochondrial body has assumed a conical shape.
 Fig. 26. Showing extension of nucleus along the plasma-rod; the central stainable cord of the latter is now visible.
 Fig. 27. Showing transverse section of mitochondrial body in same phase as Fig. 22; part of the nucleus also appears in the section.
 Figs. 28-29. Spermatozoa in transverse section, showing folding of central cord.
 Figs. 30-31. Spermatozoa; final stage of development. Fig. 30 shows a spermatozoon from the vas deferens; Fig. 31 a somewhat younger stage with centrosomal corpuscle.



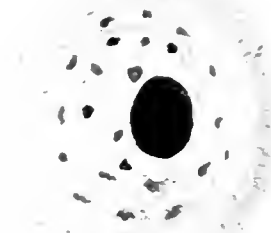
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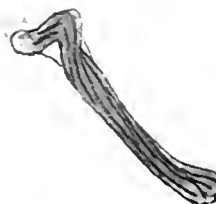
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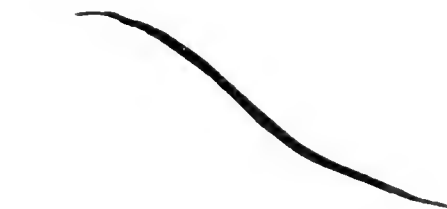
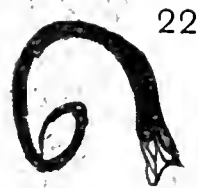
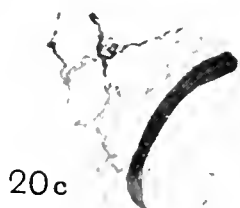
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MALLOPHAGA FROM SOUTH AFRICAN BIRDS.

DESCRIPTIONS OF A NEW GENUS (*NEOMENOPON*) AND TWO NEW SPECIES (*MACHAERILAEMUS PLOCEI*, *NEOMENOPON PTEROCLURUS*).

BY G. A. H. BEDFORD, F.E.S.

(*Entomologist, Veterinary Research Laboratory, Union of South Africa.*)

(With Plates XII and XIII.)

THE species herein described both belong to the family Menoponidae. One of these species, collected by me from a Waxbill (a passerine bird) at Onderstepoort, Pretoria, belongs to the genus *Machaerilaemus* Harrison, the other, taken from a Sandgrouse in the Rustenburg District, Transvaal, by Mr Powell, together with specimens of a species of *Degeeriella*, I am placing in a new genus, for which I propose the name *Neomenopon*. These two new species are extremely interesting, in that they both possess a chitinous framework extending backwards from the anterior margin of the head for the support of the mandibles, a structure previously only known to occur in the genus *Eomenopon* Harrison, which was established partly on account of this structure. A similar structure, however, also occurs in a small unidentified species of *Menopon* taken from a Little Banded Goshawk (*Astur polyzonoides*) at Onderstepoort. This species may eventually prove to be sufficiently distinct in other details from the type of *Menopon* to warrant the founding of a new genus for its reception.

Curiously enough, both *Eomenopon* and *Machaerilaemus* were described by Harrison in the same paper in 1915, *Eomenopon* being established for the reception of a species found on two species of Australian Lorikeets, and *Machaerilaemus* being established for a species found on an Australian Grassfinch (a passerine bird). Since then Harrison has included Carriker's *Menopon laticorpus*, described from specimens found on an Ant-bird (*Thamnophilus doliatus*), a passerine bird, in Costa Rica, in the genus *Machaerilaemus*.

Machaerilaemus latifrons possesses a dark transverse band on the forehead, which is also present in both the species described here, and likewise in the *Menopon* from a hawk, but in these the bands are inconspicuous, very short, and are interrupted in the middle.

In the type of *Machaerilaemus* the mesothorax is separated from the metathorax, whereas in the new species described here these parts are fused together.

The following table will serve to differentiate the three genera: *Eomenopon*, *Machaerilaemus* and *Neomenopon*:

(1) Head not twice as wide as long, with a deep narrow cleft in the side of the forehead, extending to the inner border of the antennary fossa; chitinous framework for support of the mandibles present, extending backwards from the anterior margin of the head to form a pair of short free projecting spinous processes; meso- and metathorax distinct. *Eomenopon*.

Head more than twice as broad as long, without a cleft in the sides of the forehead; no spinous processes attached to the chitinous framework when present.

(2) Head with a large chitinous plate on the throat, flanked by two dagger-like processes (present or absent in *M. laticorpus*?) with or without a central circular perforation; chitinous framework absent or present; meso- and metathorax distinct or fused. *Machaerilaemus*.

No chitinous plate on throat; chitinous framework present; mesothorax fused with the metathorax. *Neomenopon*.

Genus MACHAERILAEMUS Harrison.

Harrison (1915). *Parasitology*, VII. 389.

Machaerilaemus plocei, n. sp. (Plate XII, figs. 1-3).

Female. Ground colour pale brown, with darker markings of the same colour.

Head slightly more than twice as broad as long across temples. Forehead flatly rounded in front, abruptly rounded and swollen at the sides, with six hairs on each side. Eye with two minute hairs. Temples rounded, with four long hairs and several shorter ones. Occiput slightly concave, with two median hairs just inside the margin, and two more on each side, of which the outer one is the shorter. The only other hairs present on the dorsal surface are: two hairs on the forehead situated midway between the lateral angles and the middle line, the outer one being slightly longer than the inner one, and a short hair a little distance above these.

On the ventral surface the most conspicuous features are the chitinous framework, which has already been described; the gular plate and a more or less triangular plate on either side of this. The gular plate is heart-shaped, with a free projecting spinous process and five or six hairs on each side. Antennae with the two terminal segments indistinct. Palpi with the apical

segment the longest, the second and third sub-equal, and slightly longer than the first. Above the gular plate there are two hairs, and two more, slightly shorter, occur above these.

Thorax. The prothorax is winged, with two short spines, a long hair, and another short spine on each side, and a row of eight hairs on the posterior margin. The scapulars and interscapular bar are less developed than is usual in this family. On the prosternum there is a plate, and beneath this another plate, which extends on to the metasternum, the shape of these being shown in Pl. XII, Fig. 2. The metathorax has a row of about ten hairs on the posterior margin, and a long hair and several short spines at the postero-lateral angles. On the ventral surface, between the mid and hind coxae there is an inconspicuous plate clothed with shortish hairs.

Legs. The coxae are elongated; the second and third pairs each with five short spines, two being situated near the apex and three at the apex. Hind femora without a tuft of hairs or spines on the ventral surface. Mid and hind tibiae with several short spines near the apex.

The *abdomen* is elongate-oval, being broadest at the fourth segment. The tergites each with a pale brown transverse band, and a series of hairs on the posterior margin. (On each sternite there is a dark transverse band, with three irregular rows of hairs on it, and with three or four short spines at the postero-lateral margins of the bands.) The transverse band on the first sternite narrows out to a point at either end, and the bands on sternites six and seven are fused together. On the eighth sternite there is a narrow longitudinal blotch on either side of the median line, which broadens out posteriorly. The borders of the vulva are closely beset with a row of fine hairs.

The *pleurites* are dark brown, with several short hairs in the middle, and a row of longer ones and one or two spines on their posterior margins.

Male. The male resembles the female, except for the markings on the venter of the terminal segments, as will be seen by comparing Pl. XII, figs. 1 and 3, the former of which shows the ventral markings on the terminal abdominal segments.

The general form of the genitalia can be seen in Fig. 1.

Measurements in millimetres.

	Female		Male	
	Length	Breadth	Length	Breadth
Head	0.26	0.6	0.23	0.51
Prothorax	0.13	0.44	0.1	0.35
Metathorax	0.15	0.56	0.11	0.41
Abdomen	1.09	0.86	0.7	0.56
Total	1.63		1.14	

Described from one ♀ and one ♂ taken from a Waxbill at Onderstepoort, Pretoria, on 14. XII. 1918.

Genus NEOMENOPON, n. gen.

The genus may be characterised as follows: Head with distinct and fairly deep ocular emarginations; very broad, more than twice as wide as long; temples large. Prothorax with lateral margins rounded. Mesothorax fused with the metathorax. Pleurites well developed.

Neomenopon pteroclurus, n. sp. (Plate XIII, figs. 1, 2).

Female. Specimens preserved in alcohol are extremely dark, almost black, and it is then impossible to make out the markings. When mounted in Canada balsam, however, they are brown in colour, with dark brown markings. The *head* is extremely broad, being slightly more than twice as wide across the temples as long. The forehead is flatly rounded in front, and turns abruptly inwards on each side, extending backwards from the lateral angles in an almost straight line to the temples. On the margin of the forehead there are sixteen hairs, and on the dorsal surface there are six, situated in a line between the lateral angles; of these, one is situated on each side, midway between the median line and the lateral angle, and two—a long and a minute one—on each side between this hair and the margin. The temples are abruptly rounded, with about 10 long and short hairs on the margins. Occiput very slightly concave, with six hairs. Eye large, without a hair. On the ventral surface there is a chitinous structure for support of the mandibles, which consists of a band projecting backwards and slightly inwards on each side from the anterior margin to a short distance beyond the antennary fossa, where each is joined together by a transverse band. From each of the two angles formed by these bands there extends a curved band to the occipital margin. Above the transverse band there are two hairs, and above these are two shorter ones. The apical segment of the palpi is the longest, the third is the shortest, and the first and second sub-equal.

The shape of the two terminal segments of the antennae cannot be made out, owing to their being partly hidden by the pockets formed by the forehead and temples, which are very dark. On each side of the gular region there are four or five hairs in a longitudinal row.

Thorax. The prothorax is winged, with a short spine on each side in front, a long hair beneath it, and another short spine beneath this again; and on the posterior margin there are eight hairs. The interscapular bar does not quite reach the scapulars, and it is crossed by a median longitudinal bar.

The metathorax is shorter than the prothorax, with twelve hairs on the posterior margin, and a short spine on each side near the latero-posterior angles. On the metasternum there is a narrow longitudinal median plate bearing about ten hairs, and below this, between the mid and hind coxae, a median patch of nine hairs.

Legs. The coxae of the forelegs are elongated; coxae of mid and hind legs with three or four spines on or near their apical margins. The femora are very broad, and the hind pair each have a tuft of about 60 short hairs on their ventral surface.

The *abdomen* is broadly oval, being widest at the fourth segment. The tergites each have a pale transverse band (serrated on its posterior border) extending to, or almost to, the pleurites and with a series of about 18 to 20 hairs on their posterior margin. On each sternite there is also a transverse band, which is darker and not so wide as that on the corresponding tergite; on each band there is an irregular series of short hairs, which on tergites 3 to 6 form a more or less dense patch on the sides. The band on the seventh sternite is interrupted in the middle, the median space being filled by a still darker blotch, and the same appears to be the case on the sixth sternite, at least the median area is also darker than the rest of the segment. On sternites 7 and 8 there is a longitudinal blotch on each side of the median line, extending from the middle of the seventh to the apex of the eighth. The vulva is broadly rounded, with a series of fine short hairs on its margin.

The *pleurites* are well developed, brown in colour, with several hairs in the middle, and a series of longer ones and one or two small spines on their posterior margin.

Measurements in millimetres.

Female	Length	Breadth
Head	0.41	0.86
Prothorax	0.16	0.61
Metathorax	0.15	0.81
Abdomen	1.51	1.45
Total	2.23	

Described from one adult and two immature ♀♀ found on three specimens of Namaqua Sandgrouse (*Pteroclorus namaqua*) in the Rustenburg District, Transvaal, by Mr Powell in 1917.

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- HARRISON (1915). On a New Family and Five New Genera of Mallophaga. *Parasitology*, VII. 385-393.
- NEUMANN (1912). Notes sur les Mallophages, II. *Archives de Parasitol.* xv. 353-368.

EXPLANATION OF PLATES XII AND XIII.

PLATE XII.

Machaerilaemus plocei, n. sp.

- Fig. 1. Male, dorsal aspect.
Fig. 2. Male, head and prothorax, ventral aspect.
Fig. 3. Female, posterior abdominal segments, ventral aspect.

PLATE XIII.

Neomenopon pteroclurus, n. sp.

- Fig. 1. Female, dorsal aspect.
Fig. 2. Female, head and prothorax, ventral aspect.

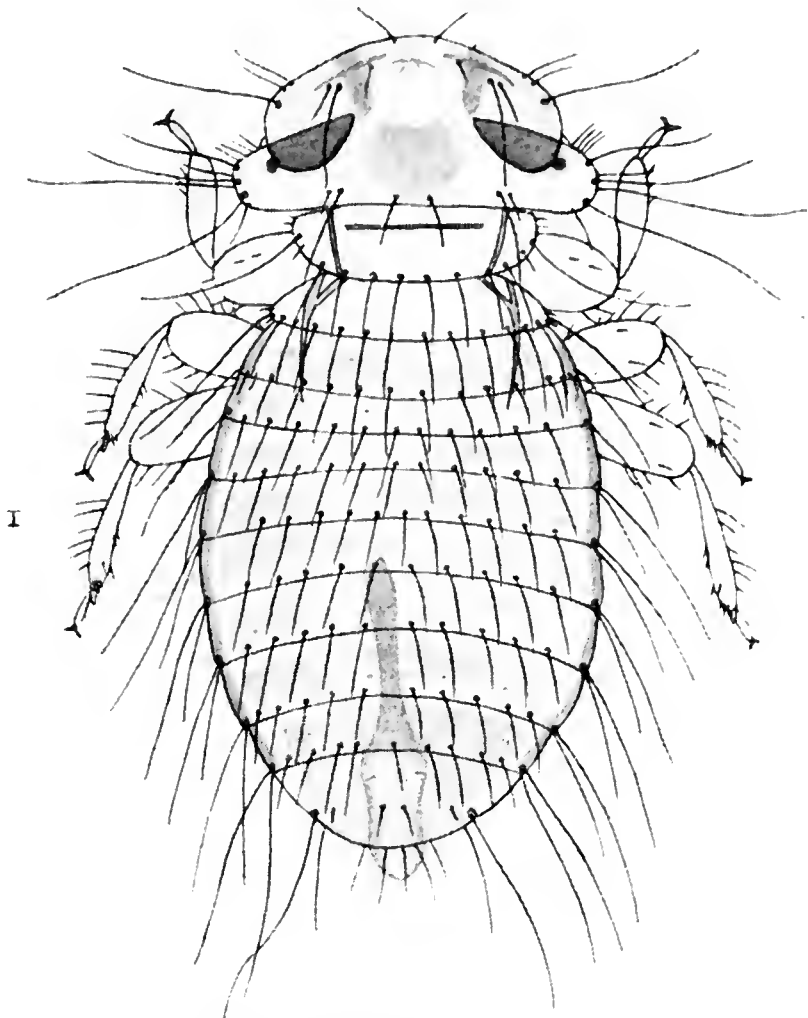


Fig. 1. *Machaerilaemus plocei*, n. sp. ♂

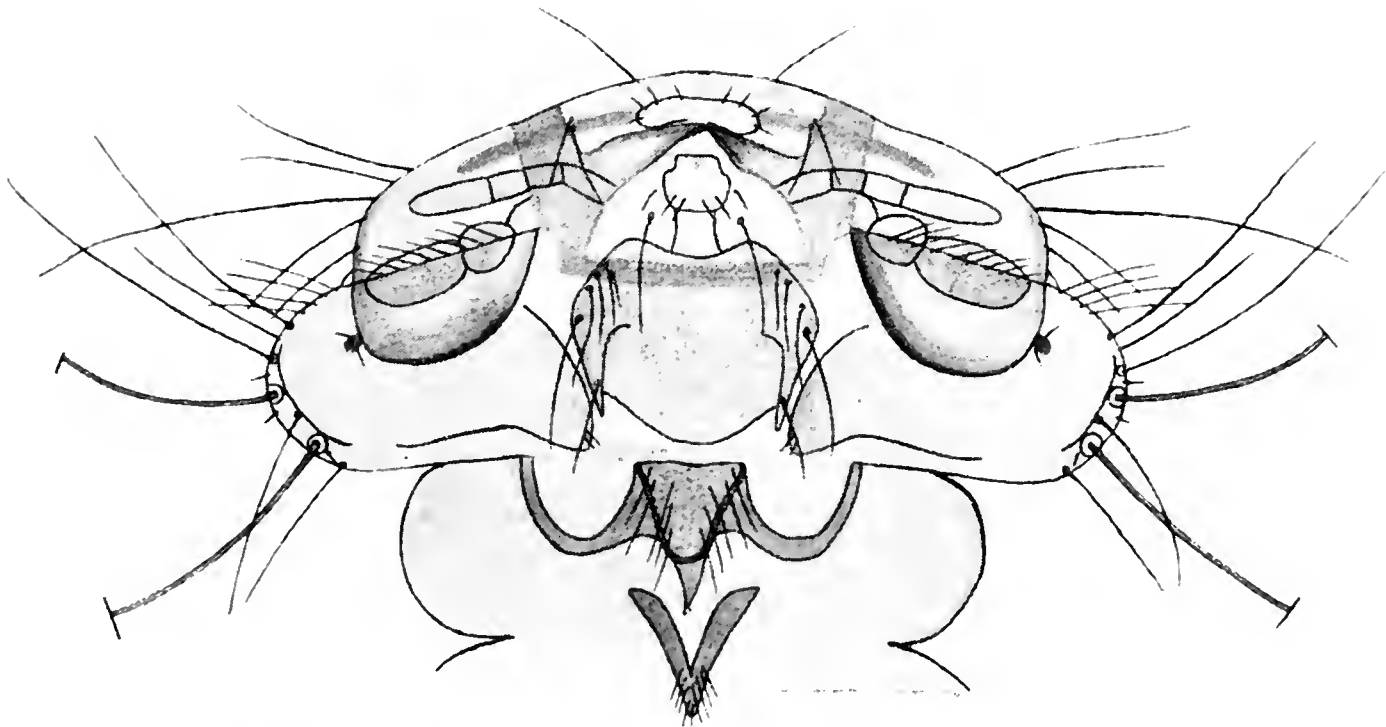


Fig. 2. *Machaerilaemus plocei*, ventral surface of head and prothorax of ♂

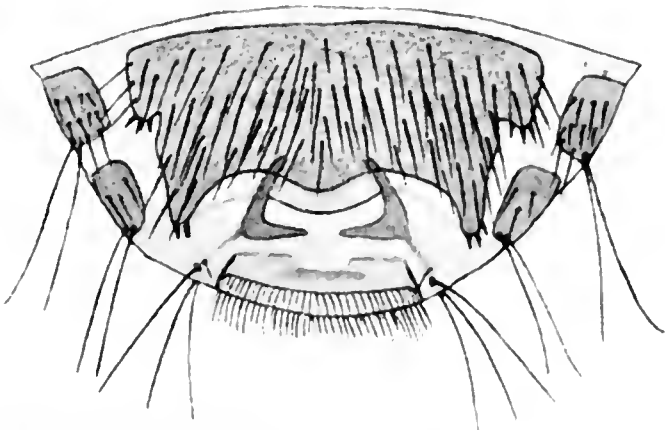


Fig. 3. *Machaerilaemus plocei*, ventral surface of terminal abdominal segments of ♀

G. A. H. B. del.

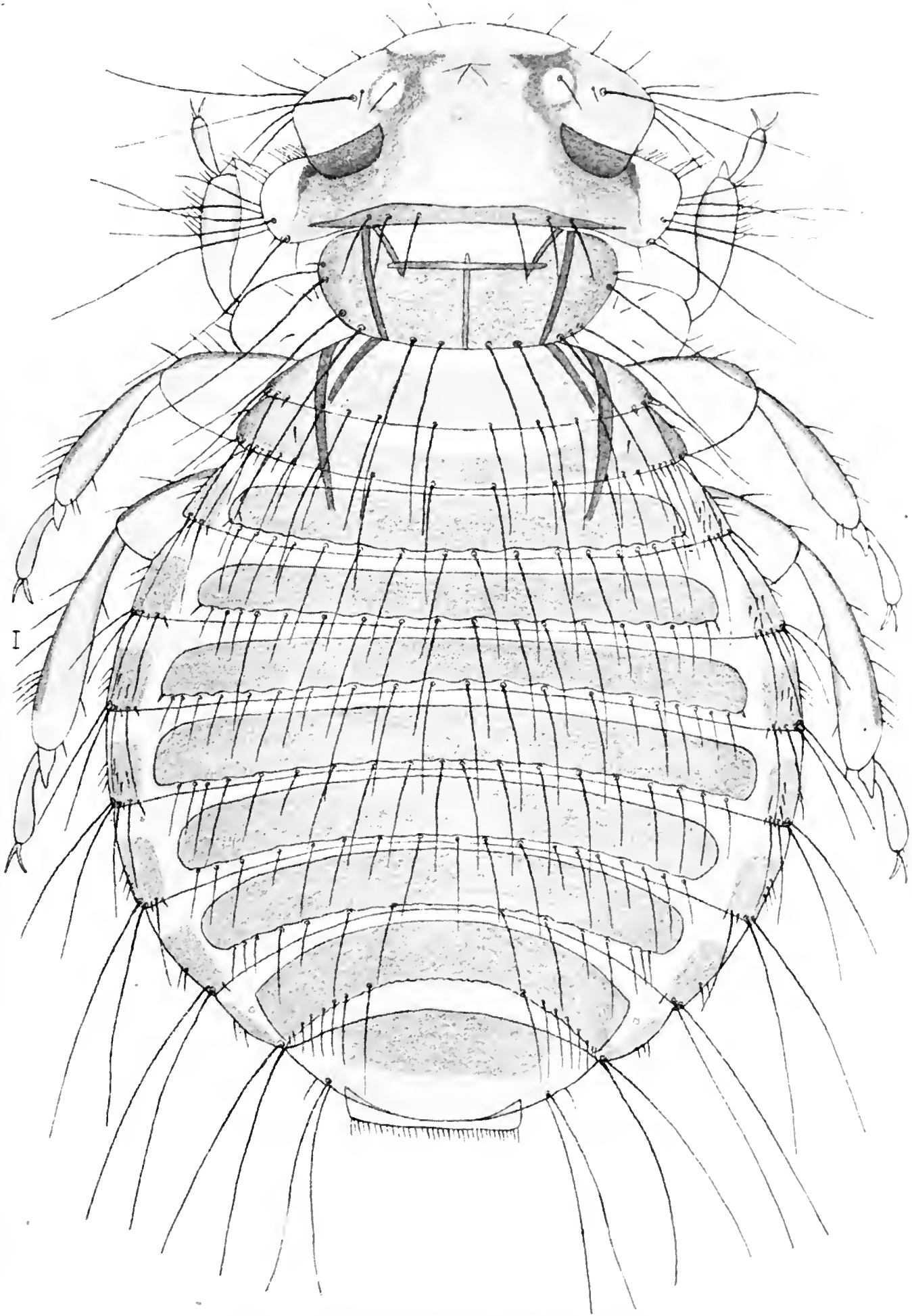


Fig. 1. *Neomenopon pteroclorus*, n. sp. ♀

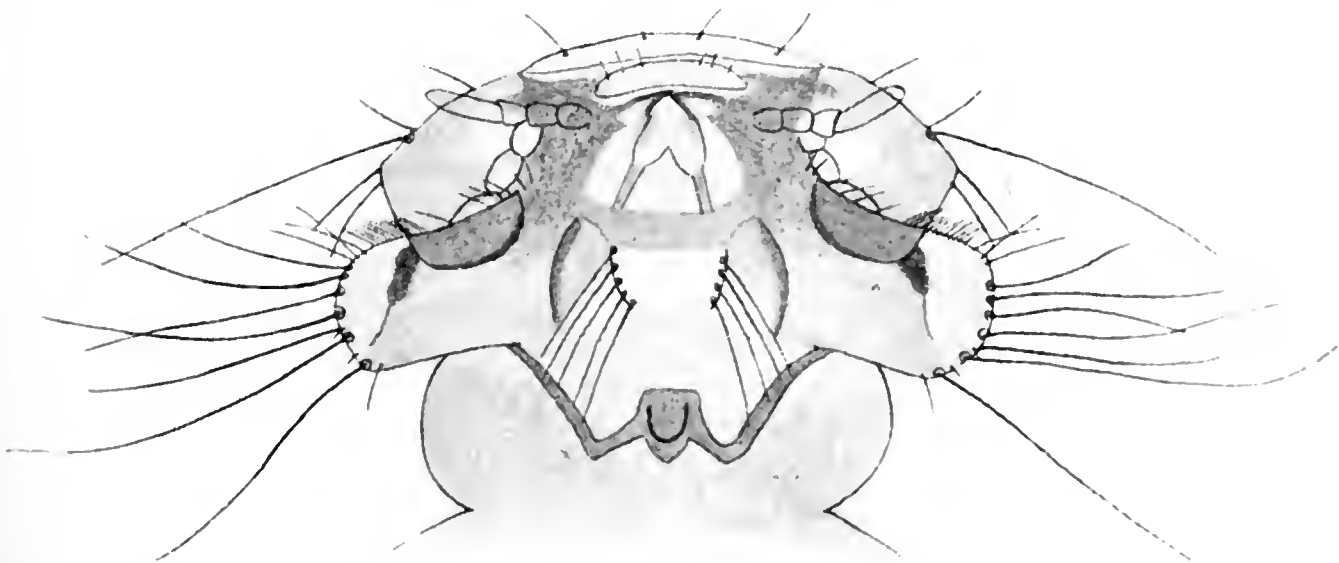


Fig. 2. *Neomenopon pteroclorus*, ventral surface of head and prothorax

BODY-LICE UNDER SUMMER CONDITIONS IN MESOPOTAMIA.

By P. A. BUXTON, M.A., M.R.C.S.

(Fellow of Trinity College, Cambridge.)

DURING the summer months in Mesopotamia body-lice become extremely scarce on man, so much so that it may be difficult to find them even on men who are renowned for lousiness at other seasons of the year. When the nights become cold, as they often do rather suddenly towards the end of November, lice rapidly become numerous, this coinciding with the reissue of winter under-linen which has been stored through the summer in Ordnance dumps.

During my service as Entomologist to the Mesopotamian Force I constantly encountered officers, specialists in sanitation among them, who erroneously supposed that the lice survived the unfavourable conditions in summer as eggs in the stored warm clothing, the Ordnance being in fact blamed for distributing verminous clothing to various highly respectable units who would otherwise, as I was assured, not have suffered from these pests. I always pointed out that this was impossible in view of what we know of the duration of the egg and other stages of this insect, but as superstitions die hard it may be as well to record that during the summer of 1919, in the Persian plateau, which enjoys a summer as dry as that of Mesopotamia and only a little cooler, I could always find body-lice on 5 per cent. or 10 per cent. of Indian troops, even in one excellent unit the men of which were inspected by their own officers every week. In these dry hot summers lice appear to survive with difficulty and to breed very slightly. On the men in question I rarely found more than one or at the most two lice and this explains why their own officers failed to find them.

The Mesopotamian summer, which appears so unfavourable to reproduction, is generally very dry. Maximum shade temperatures pass 120° F. once or twice every summer, and pass 105° F. most days in the four summer months. Under active service conditions men were habitually exposed to higher temperatures than these official ones. In N.W. Persia, at Qazvin,

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the official reading rarely exceeded 100° F. but ran up in tents to 115° F. most days for about two months. The climate was extremely dry.

This note may appear to entomologists to repeat what is known¹, but I publish it in the hope that it may perhaps help to convince that "practical man" the sanitarian.

¹ Consult Nuttall (*Parasitology*, x, pp. 87-91, 132; xi. pp. 205, 206) on seasonal variation in the number of lice on man.

MALFORMATIONS IN TICKS.

BY L. E. ROBINSON, A.R.C.Sc. (LOND.).

*(From the Quick Laboratory, University of Cambridge.)**(With 5 Text-figures.)*

IN 1909, and again in 1914, papers, recording cases of deformity in Ticks, were contributed to this journal, by Warburton and Nuttall¹, and Nuttall², respectively: since that time, numerous examples of malformation have been observed in the material examined by them and by myself, in the course of our collaboration in work on Ticks in general. Of these examples, the five which are described and figured below, are sufficiently striking to be placed on record. The figures have been drawn with the aid of the Abbe-Zeiss camera lucida, and, in each case, the degree of magnification is indicated by a scale representing a length of one millimetre.

Specimen 1.

Dermacentor atrosignatus Neumann, 1906, ♂, taken off a dog, at Kosempo, Formosa, collector's name not recorded. The specimen was received, on loan, from Prof. L. G. Neumann, and was derived, in the first instance, from the collection of the Deutsches Entomologisches Museum, Berlin. With the exception of the hypostome, the tick is normal in all respects. The hypostome shows a considerable degree of asymmetry, due to an antero-mesial displacement of the denticles on the left side. The non-denticular proximal portion exhibits an anomalous feature, in that it bears three forwardly-directed salient points; these are clearly displayed in the figure.

Specimen 2.

Amblyomma hebraeum Koch, 1844, ♀ (N. 2413 a). The specimen was found on a cow (28. VII. 1913), at Namahacha, Portuguese East Africa, and is the gift of Dr J. B. Botelho. The scutum is not only deformed, but is remarkably small for the species, its length measuring barely two-thirds of the length of a normal example. The eye is absent on the left side, and, immediately

¹ Warburton, C. and Nuttall, G. H. F. (1909), On new species of Ixodidae, etc., *Parasitology*, II. 57-76.

² Nuttall, G. H. F. (1915), Tick abnormalities, *Ibid.* VII. 250-257.

posterior to the site which it should occupy, the scutal margin is eroded by a striated cicatrix, which extends in a postero-lateral direction over the general

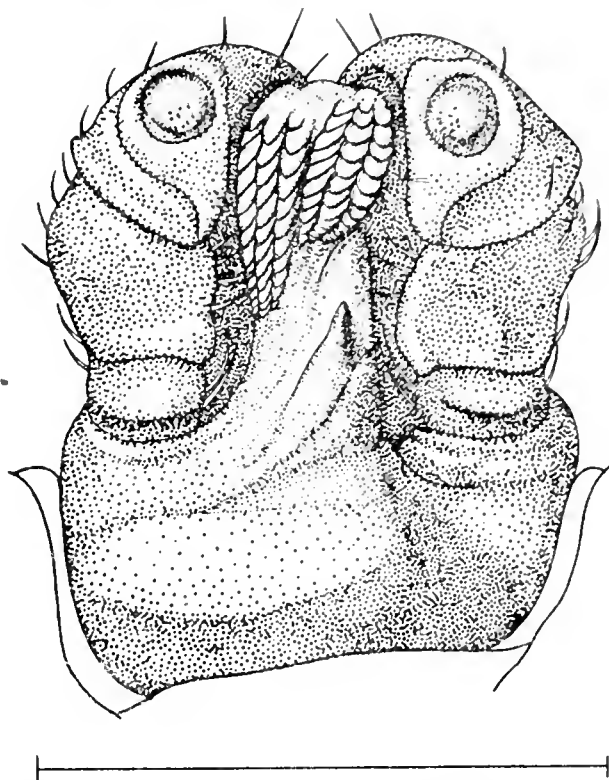


Fig. 1. *Dermacentor atrosignatus* ♂. Abnormal: deformity of the hypostome. (L.E.R.)

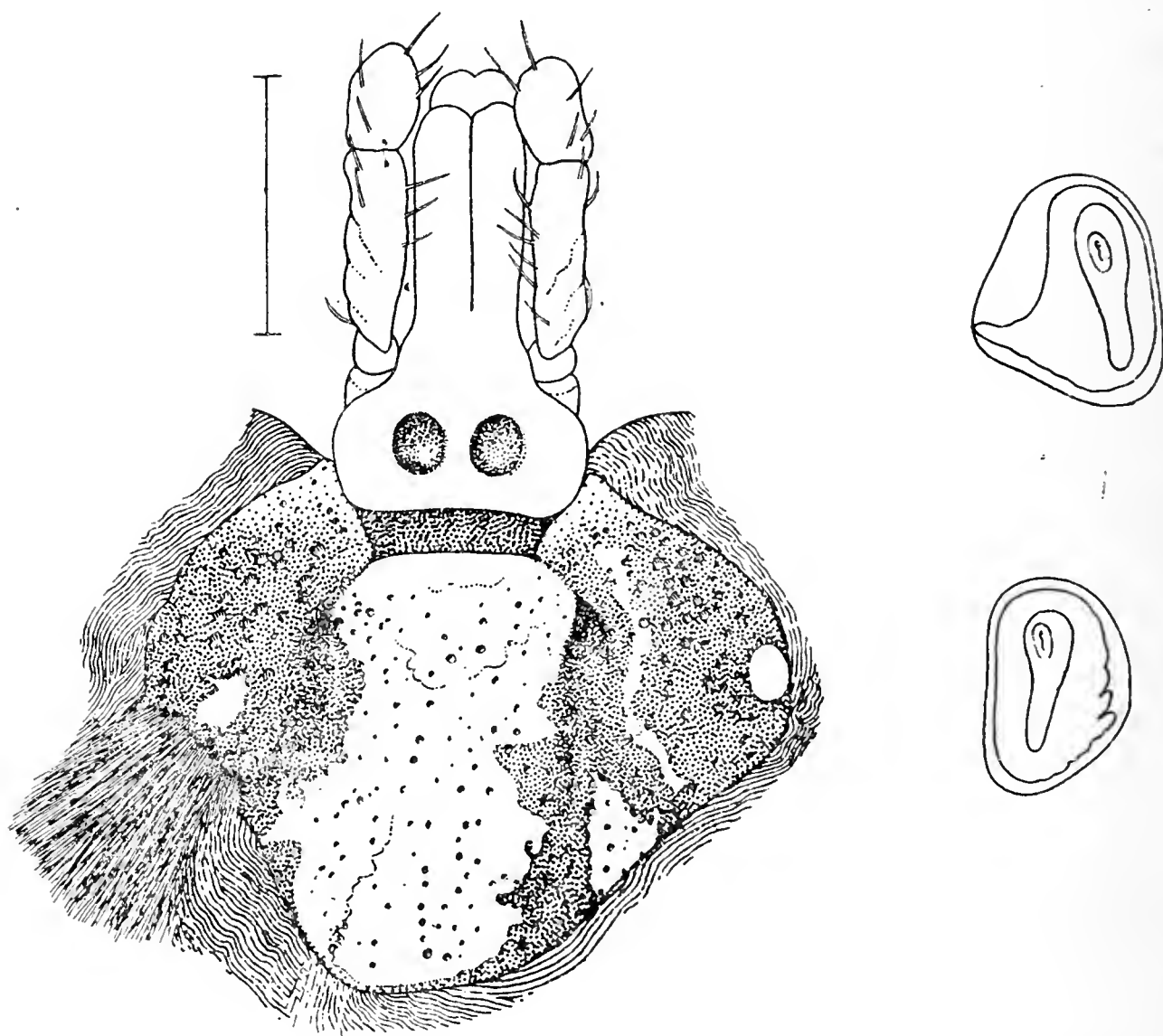


Fig. 2. *Amblyomma hebraeum* ♀. Abnormal: absence of left eye, and deformity of scutum and spiracle. (L.E.R.)

body integument. The left spiracle, although widely separated from this cicatrix by an extent of normal cuticle, is also deformed (see the lower of

the two figures of the spiracle; the uppermost figure represents the right spiracle of the specimen, which presents a normal appearance).

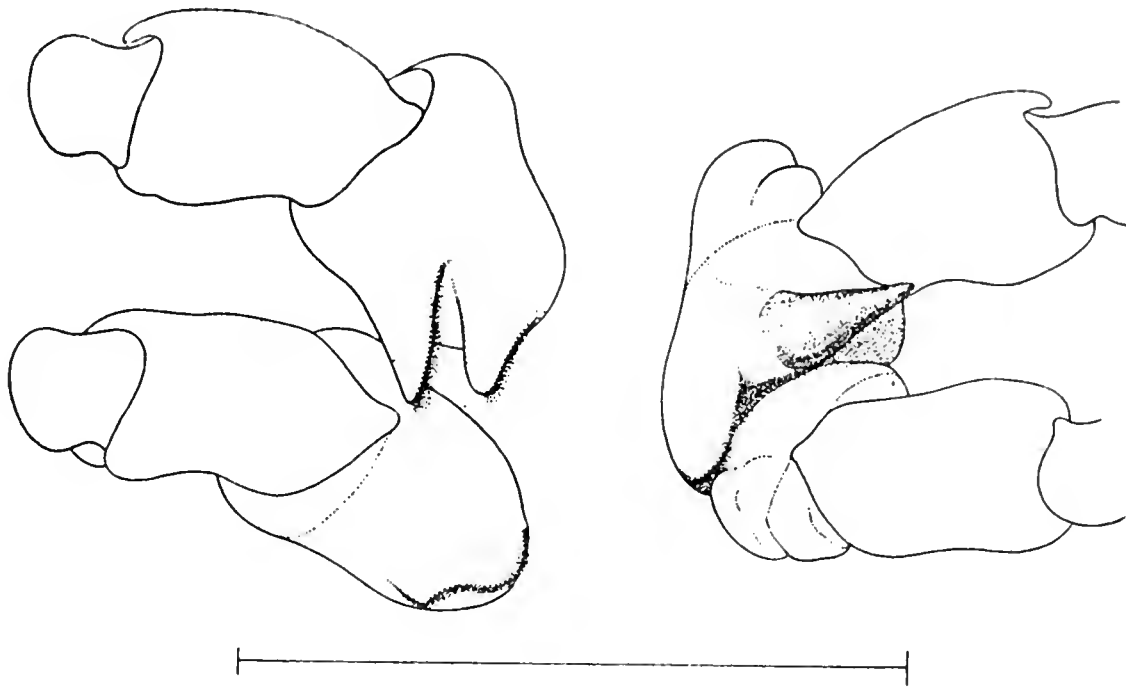


Fig. 3. *Amblyomma cajennense* ♂. Abnormal: fusion of coxae i and ii, with suppression of normal coxal armature. (L.E.R.)

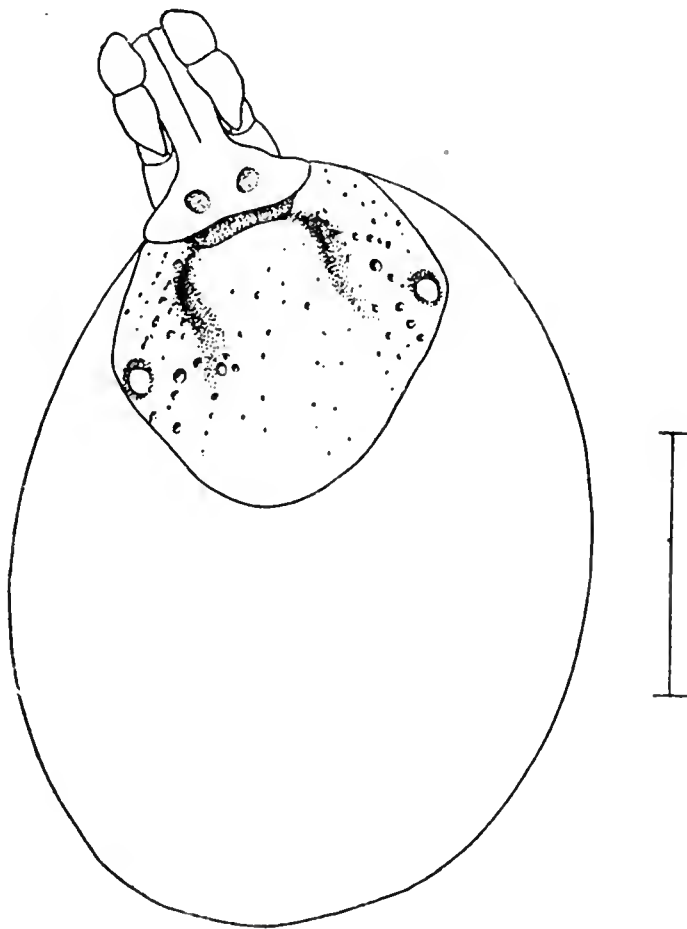


Fig. 4. *Hyalomma aegyptium* ♀. Abnormal: oblique curvature of body axis. (L.E.R.)

Specimen 3.

Amblyomma cajennense (F.), ♂ (ex N. 1654). This specimen was included with examples of *Ambl. coelebs* Nn., in a tube of ticks received from the Liverpool School of Tropical Medicine, labelled "No. 144," but with no other data. The specimen shows a fusion of the 1st and 2nd coxae on the left side:

the coxal armature of both is reduced to a single, stout, conical spur, which projects almost perpendicularly from the ventral surface. The corresponding coxae of the opposite side are normal, and are figured for the purpose of comparison.

Specimen 4.

Hyalomma aegyptium (L.), ♀ (N. 3347), off *Hemitragus hilocrius*, Cochin State, Madras, India; received from the Indian Museum, Calcutta. The specimen presents a considerable degree of asymmetry, due to an oblique curvature of the axis of the body. This is the most frequently occurring type of malformation in ticks. The fact that the scutum is also malformed, would appear to exclude the possibility that the distortion has been caused by unequal distension during the act of engorgement, a cause to which the condition often may be attributed.

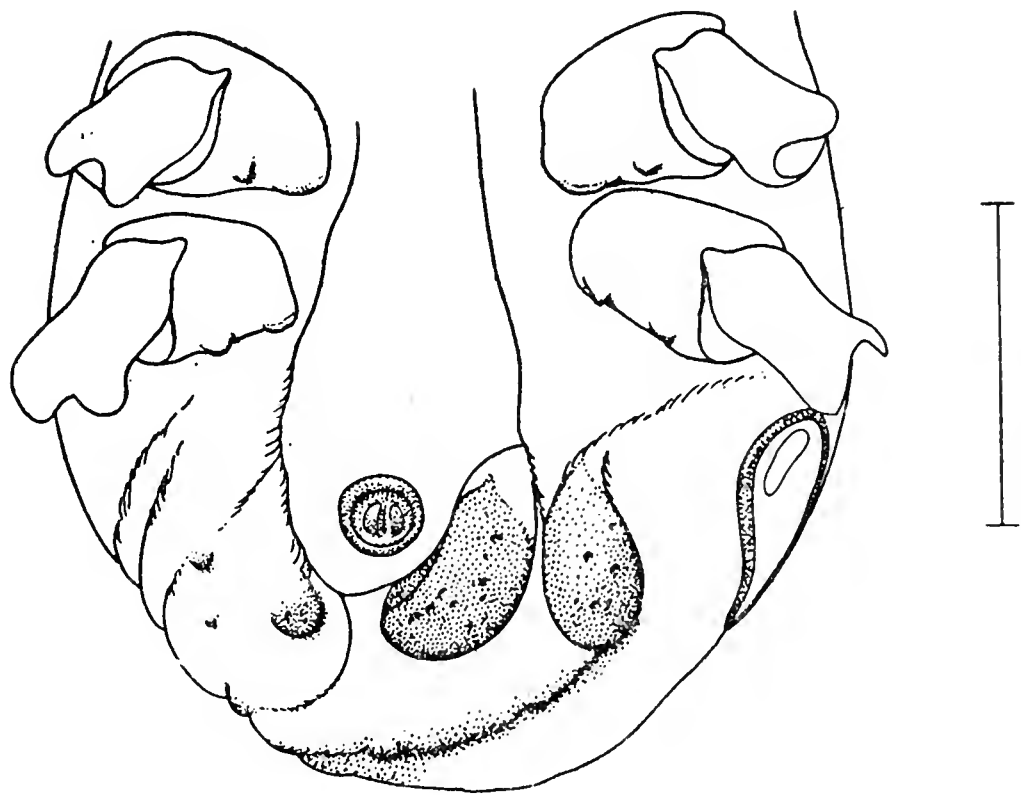


Fig. 5. *Hyalomma aegyptium* ♂. Abnormal: suppression of spiracle and adanal shields on right side of body. (L.E.R.)

Specimen 5.

Hyalomma aegyptium (L.), ♂ (N. 26 c), off goat, Germiston, Transvaal, S. Africa, 6. iv. 1918. The specimen was sent by Dr B. G. Brock. The posterior portion of the body is asymmetrical; the right spiracle is completely absent, and the adanal shields are reduced to vestiges on the right side. On the left side of the body, all of these structures are normal.

Such malformations as those described above, are probably the result of some mutilation of the tick during the course of the preceding nymphal phase. Experimental investigations of the power of regeneration of injured or amputated appendages, as manifested by ticks, have shown that this

power is developed to a high degree in the Ixodid ticks¹ especially, and to a smaller degree in the Argasid ticks². In the course of engorgement, particularly as the state of repletion is approached, the chances of injury to which a tick is exposed are numerous, owing to the intentional or accidental scratching and rubbing of the infested parts of its body by the host, and the remarkable power of regeneration of damaged or lost appendages which the Ticks, in common with some other Arthropods, have developed, must ensure the survival of many individuals which otherwise would perish.

¹ Nuttall, G. H. F. (1920), Regeneration in Ticks. *Parasitology*, xii. 7-26.

² Hindle, E. and Cunliffe, N. (1914), Regeneration in *Argas persicus*. *Ibid.* vi. 353-371.

A MALARIA SURVEY IN THE MALAY ARCHIPELAGO.

BY N. H. SWELLENGREBEL, PH.D.

AND J. M. H. SWELLENGREBEL-DE GRAAF, *Amsterdam*.*(From the Institute of Tropical Hygiene, Amsterdam.)*

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INTRODUCTION.

THE great discovery of Sir Ronald Ross has not led to the suppression of malaria in the Netherlands' East Indian Colonies, where the disease is still all too prevalent. The failure is partly, if not wholly, due to the mistake that has been made in considering the facts revealed by this discovery as an all sufficient basis for practical sanitary measures, whereas they can only serve as a foundation for further research in which special and local conditions are taken into account, with the object of acquiring information which is essential for the success of a sanitary campaign.

Local research has been much hampered by the confusion which has existed in the nomenclature of the various Anopheline species of the Malay Archipelago, a confusion due, no doubt, to entomologists, notably Dönitz and Theobald, having worked at the subject more or less independently. This lack of coordination gave rise to a confusion which rendered the subject almost unapproachable for the ordinary medical man. Apart from specimens preserved in European museums, nothing was known concerning our Anopheline fauna, and especially notable was our ignorance regarding the infectability of the various species with malarial parasites. Ignorance of these essential data made successful anti-malaria measures impossible because we undertook the impossible task of abolishing all Anopheline breeding places. The number, variety, and extensive distribution of these breeding places, including waggon tracks, hoof-prints, and other small depressions, of constant occurrence, even on well drained land, during the rainy season, made effective petrolisation impracticable. Could this difficulty have been surmounted, there still remained the most extensive breeding places of all, the rice fields. These furnish the

staple food for over thirty millions of inhabitants, and can neither be abolished nor petrolised.

The problem offered little prospect of solution, until we became acquainted with Watson's results (1911) in the Federated Malay States. His campaign was preceded by a thorough systematical and biological investigation of the local Anopheline fauna, and from the knowledge thus acquired, it was possible to conduct repressive operations against selected species.

Encouraged by Watson's success, Schüffner and Swellengrebel (1914) began to study the Anopheline fauna of the Malay Archipelago, with the result that our knowledge of the local species is now fairly complete¹.

The Anopheline genera and species of the Malay Archipelago are as follows:

GENUS.	SPECIES.
<i>Myzomyia</i> .	<i>ludlowi</i> , <i>rossii</i> , <i>indefinita</i> , <i>minima</i> , <i>minima</i> var. <i>aconita</i> , <i>flava</i> *.
<i>Neomyzomyia</i> .	<i>leucosphya</i> , <i>punctulata</i> (<i>tesselata</i>).
<i>Nyssorhynchus</i> .	<i>fuliginosus</i> , <i>maculatus</i> , <i>jamesii</i> *, <i>schüffnerii</i> *, <i>annulipes</i> var. <i>moluccensis</i> *.
<i>Cellia</i> .	<i>kochii</i> .
<i>Myzorhynchus</i> .	<i>gigas</i> , <i>sinensis</i> , <i>sinensis</i> var. <i>vanus</i> , <i>sinensis</i> var. <i>separatus</i> , <i>barbirostris</i> , <i>barbirostris</i> var. <i>pallidus</i> *, <i>umbrosus</i> , <i>umbrosus</i> X (Watson), <i>albotaeniatus</i> , <i>mauritanus</i> *.
<i>Stegomyia</i> .	<i>aitkenii</i> , <i>aitkenii</i> var. <i>insulae florum</i> *, <i>aitkenii</i> var. <i>papuae</i> *.

The species marked with an asterisk are not recorded from the Malay Peninsula, while the following species have not hitherto been found in our Colonies:

Mennemyia brevipalpis, *Lophoscelomyia asiatica*, *Pyretophorus watsonii*, *Myzomyia aurostris*, and *Myzorhynchus albotaeniatus* var. *montanus*.

Reverting to Watson's researches mentioned above, we find that in the hills of the Malay Peninsula, many difficulties have still to be overcome, but in the lowlands sanitary measures have become comparatively easy, as a result of these researches. Of the Anophelines found in the Malay Peninsula, only *Myzorhynchus umbrosus* is an important vector; its larva is peculiar, in that it only breeds in pools in the jungle. If these pools were abolished, malaria would be abolished, without incurring the expense and trouble which would be necessary in a general campaign directed against all species of Anophelines. This instance shows that eradication measures directed against selected species are profitable only under the following conditions:

- (a) that one, or a very limited number of species are important vectors,
- (b) that their breeding places are particular and restricted.

In the following pages we shall use the term "specific sanitation" to define anti-malarial measures directed against one or a few species, to the exclusion of those which are non-carriers or unimportant carriers. In drafting our programme of research in the Malay Archipelago, it was essential to discover:

¹ Schüffner and Swellengrebel (1914); Schüffner and Van der Heyden (1917); Swellengrebel (1914), (1916), (1917), (1918 b), (1919 a); Swellengrebel and Swellengrebel-de Graaf (1919 b); Winoto (1918).

- (1) The species of Anophelines that are the principal vectors.
- (2) The habitats of the larvae of these Anophelines.

Although it has been shown that the Anopheline fauna of the Peninsula and Archipelago are practically identical, we had doubts from the start, as to the advisability of applying without further investigation the results obtained in the Peninsula. This cautious attitude has proved to be justified.

In the following we shall constantly allude to the results of other investigators in the Malay Archipelago—Schüffner, v. Breemen, Bais, Citroen, and Winoto.

I. The Principal Vectors of Malaria in the Malay Archipelago.

At the commencement of our inquiry (February, 1917), data relating to this subject with respect to our Colonies, were scanty. Schüffner (1902) and de Vogel (1909) had succeeded in infecting mosquitoes, but unfortunately some doubt exists as to the species with which they experimented. We regard it as highly probable that Schüffner experimented with *M. ludlowi*, and de Vogel with *M. rossii*. As regards the Malay Peninsula, our information was more extensive, comprising, as it did, the results of the researches of Watson (1911), Stanton (1914), and Strickland (1916). We are also acquainted with the work of Walker and Barber (1914) in the Philippine Islands, and with that of Kinoshita (1906) in Formosa. Barber's paper (1918) made its appearance some months after the publication of our first paper. The authors I have cited indicate *M. umbrosus*, *N. maculatus*, *M. aconita*, *M. minima* (*febrifer*), and *M. sinensis* as the most dangerous species, but none of them appears to consider *M. ludlowi* as an important vector, although Christophers long ago proved it to be such in the Andaman Islands. Stanton is dubious on the subject, while Watson (1915, pp. 82–83) remarks "My feeling from what I have seen in the Federated Malay States is, that *A. ludlowi* can exist, without producing malaria, and indeed that malaria will disappear from a place where steps are taken which abolish only *A. umbrosus*—a proved carrier, yet leave very large numbers of *A. ludlowi*." Strickland considers it to be of importance, but only if it is very plentiful, contrary to the hill species which remain dangerous even when scanty. These opinions are interesting in the light of our own findings, which showed *M. ludlowi* to be the most important vector of all the Archipelagan species. This shows the indispensability of local researches. Had we neglected them by simply accepting the results obtained in neighbouring countries, we should have fallen into grave errors.

Whether or not a certain species is a good vector may be determined by (a) the direct and (b) the indirect method. For the present we shall consider the direct method only. This consists in determining the rate of infection with malaria parasites (a) in mosquitoes fed on gamete carriers (rate of experimental infectability or shortly "E. I.") or (b) in mosquitoes caught in nature (rate of natural infectability, in short "N. I."). Of the two methods, the first has found a much larger application, because it is more generally practicable.

It is often extremely difficult to procure mosquitoes in numbers sufficient for the determination of the N. I.; and, moreover, if they are found to be all uninfected, it may still be asked whether there was no flaw, and whether the species under examination is not after all a good vector. In experimental work no such doubt exists, provided the gamete carrier is a good one, and that another species, known to be a good vector, is used as a control. It is also easier to obtain large numbers of mosquitoes artificially bred than caught in nature. On the other hand, there are many factors which determine the chance of the mosquito becoming infected, and which are not taken into account in experimental work, and so do not influence the E. I., whereas they may materially affect the N. I. In this way it may happen that the determinations of E. I. and N. I. yield different results. Thus we found this to hold for *M. sinensis*, which was easily infected experimentally, but uniformly showed a low N. I. In this connection we may quote the observations recorded by Stephens and Christophers, and by James, which revealed a similar discordance between the N. I. and the E. I. of *M. rossii*. As to which of the two, the N. I. or the E. I., should be trusted in practice, the above-named observers unhesitatingly express a preference for the N. I. and we agree with them. Experiments show that under favourable conditions certain species may transmit malaria, but they leave us in doubt as to whether these conditions obtain in nature. Our observations may leave us uncertain whether under changed conditions a given species will or will not become a good vector, but at least they tell us that under the conditions existing in a given country, a particular species actually does or does not harbour malaria parasites. If such observations carried out in various localities, yield uniform results, the latter, in our opinion, are of more practical value than those obtained by experiment. *We have therefore adopted the determination of the N. I. as our standard method to discover the principal vectors of malarial parasites*, and, according to the conditions formulated above we have extended our researches to various localities.

The figures representing the N. I. of one and the same species in different places, all of them highly malarious during the period of observation, often show a marked divergence (Table III). One of the principal causes of this is the fact that gamete-carriers are more numerous and potent in regions of epidemic malaria, than in countries where endemic malaria prevails. Where there is endemic malaria, the spleen-rate is much the same in both children and adults, and it is invariably high (80 per cent. and more). The parasite-rate, in young children especially, is considerably higher than in adults. Comparing the rate of infection in children and adults, with the three species of malarial parasites separately, it appears that in adults, the three species do not disappear at the same rate, the decrease of simple tertian and quartan being more marked than subtertian. In the case of subtertian parasites, a striking feature may be noted, namely, that the decrease of crescents is greater than that of rings and the number of gametes present in the crescent-

carriers is often so small as to make it improbable that an Anopheline will become infected by the carriers' blood. In children, especially when young, both the rate of crescent-carriers and the number of gametes per carrier, are much higher.

In regions of epidemic malaria, during the course of an epidemic, the spleen-rates in children and adults are equal, and often reach a figure not less than that of the splenic index in endemic regions; but in this case, the parasite-rates in children and adults are likewise equal. No difference is to be noted in the rate of infection with the three species of malarial parasites, or in the percentage of crescent-carriers, or in the number of crescents or rings per carrier. The total number of infections is also much higher than in endemic regions.

Schüffner (1919 *a*) explains this difference by the immunity that is generally supposed to develop in a population continually exposed to malarial infection. In endemic regions this immunity is attained by the adults; in epidemic regions it is not attained because serious epidemics are generally too infrequent to allow of a lasting immunity being acquired.

The foregoing description of the relative parasitaemia in regions of endemic and epidemic malaria, is based on the most typical cases. It is obvious that, apart from these, numerous intermediate cases are to be met with, as may be gathered from the following table (Table I).

Table I.

Locality	Author and year of publication or delivery of report	Children or adults	Percentage of people examined showing infections of:				Spleen-rate
			Simple tertian	Quartan	Sub-tertian	Crescents	
Tegal (endemic)	Swellengrebel, 1919 <i>c</i>	child	8	3	32	15	91
		adult	0	0	17	1.7	91
Mandailing (endemic)	Schüffner, 1916-1917	child	6	11	25	9	96
		adult	0.3	1	5.2	0.9	96
Semarang (endemic)	Swellengrebel, 1918 <i>d</i>	child	8	4	15	10	80
		adult	2.7	0.8	7	2.7	74
North Soendatar (epidemic)	Swellengrebel, 1918 <i>e</i>	child	21	9	68	48	90
		adult	11	4	53	34	87
Naras (epidemic)	Schüffner, 1919 <i>b</i>	child	35	3.5	65	40	84
		adult	24	2.3	55	36	70
South Soendatar (epidemic)	Swellengrebel, 1918 <i>c</i>	child	4	2	18	13	21
		adult	4	3	18	13	29

In regions of epidemic malaria, the number of carriers from which Anophelines can acquire infection is much greater, because of the many infected children *and* adults. In endemic regions the number of carriers is less, these being for the most part children alone. This should be taken into account when comparing the N. I. of the same species in different localities. Species usually exhibiting a very low N. I. may, in the epidemic regions, appear to be as heavily infected as good vectors in endemic regions. This is shown in the following table (Table II).

Table II.

Locality	<i>M. ludlowi</i>			<i>M. rossii</i>			Crescent rate	
	Examined	Infected	N. I.	Examined	Infected	N. I.	Adults	Children
Panggoeng	100	16	16	93	3	3	17	32
Pendjalan	185	10	5	243	1	0.4	1.7	15
Mandailing	4609	102	2.2	—	—	—	0.9	9

The rule of the N. I. being high in regions of endemic malaria, is not without exceptions. In Soendatar this figure was less than 1 per cent. in *N. fuliginosus* and *M. sinensis*, and zero in other species, such as *M. aconita* and *N. maculatus*. In Naras, Schüffner found 6 per cent. of *M. ludlowi* infected, whereas in other endemic regions we occasionally found as high a rate as 35 per cent. for the N. I. of this species. From this we infer the existence of still other factors, whose nature is as yet unknown, which influence the N. I.

When comparing the results of determinations of N. I. in different localities, much circumspection is necessary, unless a control, in the form of a known carrier, enables us to check our findings. The best species for control purposes is *M. ludlowi*, the only mosquito which is invariably found infected in all malarious districts.

In the following tables we have collected the results of several determinations of N. I. and E. I. together with records of blood and spleen examinations. In both sets of observations, it may be noted that at least one species of mosquito was found to be infected. We have excluded all observations in which this was not the case. For a comparison of the results obtained in the Malay Archipelago with those from neighbouring countries, we have added to these tables the figures of N. I. from Malacca and British North Borneo, and the figures of E. I. from Malacca.

Supposing the N. I. of a given species to have been determined in various districts, likewise the nature of the parasitaemia of the inhabitants, is it permissible from these data to draw any conclusions as to the economic importance of this species? As a rule we may do so provided the species is found infected in every locality, and that it shows a comparatively high degree of N. I. But, this condition is seldom fulfilled (excepting *M. ludlowi*). In the majority of cases the determination of N. I. still leaves some doubt as to the significance, from the sanitary standpoint, of the mosquito under observation. As an instance, in the district of Mandailing, we found that *M. ludlowi* showed the highest N. I.; but other species were likewise infected, *M. barbirostris* especially. What degree of importance is to be attached to this species? To find an answer we must look to neighbouring non-malarious regions, where *M. ludlowi* is absent, but where many of the species, which in Mandailing showed malarial infection, are present, notwithstanding a low N. I. Our experience in other localities has taught us that severe malaria never occurs with *M. barbirostris* as the only vector. From these three data we can infer

Table III. Showing result of determination of N.I. in various localities of Java and Sumatra compared with similar figures from neighbouring countries.

Locality	Observed by	M. ludlowi			M. sinensis			M. barbirostris			M. indefnita			M. rossii			N. fuliginosus			N. punctulata			C. kochii		
		Dissected	Infected	Percentage of infection	Dissected	Infected	Percentage of infection	Dissected	Infected	Percentage of infection	Dissected	Infected	Percentage of infection	Dissected	Infected	Percentage of infection	Dissected	Infected	Percentage of infection	Dissected	Infected	Percentage of infection	Dissected	Infected	Percentage of infection
Naras (Sumatra)	Schüffner, 1919 b	66	4	6.1	388	.	.	13	.	.	97	9	.	.	49	.	.	45	.	.
Angoli (Sumatra)	" 1918 b	.	.	.	291	.	.	4	.	.	12	9	.	.	18	.	.	143	3	2.1
Western Java	Mangkoe Winoto, 1918	435	14	3.2	7	.	.	9	.	.	5	2
Batavia (Java)	v. Breemen, 1918-19	5120	82	1.6	.	.	.	9	.	.	34	.	.	2625	7	0.3	1	.	.	56	.	.	7	.	.
Siantar (Sumatra)	Bais, 1919
Sigiran (Sumatra)	Swellengrebel, 1918 a	6	1	16.6
Belawan (Sumatra)	Swellengrebel and Schüffner, 1919	679	34	5.0	1	.	.	544	3	0.5	2352	1	0.04	.	.	.	87	.	.	1380	.	.	2	.	.
Mandailing (Sumatra)	id.	4609	102	2.2	3606	4	0.1	23	.	.	12	.	.	141	1	0.7	1	.	.	1	.	.	474	.	.
Kepetakan (Java)	id.	177	63	35.0	273	1	0.4	23	.	.	53	.	.	573	3	0.5
Soerabaia (Java)	id.	740	69	9.3	115	569	2	0.3	10	.	.	66	.	.
Soendatar (Sumatra)	id.	.	.	.	701	1	0.1	6	337	4	1.2
Tegal (Java)	Swellengrebel, 1919 c	258	26	10.0	.	.	.	43	.	.	741	1	0.1
Semarang (Java)	" 1918 d	1004	41	4.1	300	1	0.3	757	5	0.7
Modjowarno (Java)	" 1919 d	.	.	.	3	.	.	31	3	.	.	13	.	.	139	1	0.7	62	.	.
Soerabaia (Java)	Reylingh, 1918	187	2	1.1
Mandailing (Sumatra)	Schüffner, 1916	30	2	6.7
Grand total		13124	438	3.3	5270	6	0.1	682	3	0.4	3721	3	0.08	4898	22	0.4	691	2	0.3	1553	1	0.06	799	3	0.4
Fed. Malay States	Stanton, 1914	.	.	.	87	2	2.4	16	.	.	114	23	1	4.3	.	.	.	7	.	.
id.	Barber, 1918	18	1	5.5	22	.	.	14	.	.	200*	4	.	.	3	.	.	6	.	.
id.	Watson, 1911
id.	Strickland, 1916	80	.	.	1	13	1
Brit. N. Borneo	Roper, 1914	8	.	.
Grand total		98	1	1.0	110	2	1.8	30	.	.	314*	.	.	13	.	.	27	1	3.7	4	.	.	21	.	.

* Mixed rossii and indefnita.

Locality	Observed by	<i>M. aconita</i>			<i>N. maculatus</i>			<i>N. karwarii</i>			<i>N. leucosphyræ</i>			<i>M. umbrosus</i>			<i>M. albotæ-niatus</i>			<i>S. aitkenii</i>			children			Examination of adults		
		Percentage of infection			Percentage of infection			Percentage of infection			Percentage of infection			Percentage of infection			Percentage of infection			Percentage of infection			Percentage of infection			Percentage of infection		
		Dissected	Infected		Dissected	Infected		Dissected	Infected		Dissected	Infected		Dissected	Infected		Dissected	Infected		Dissected	Infected		Number examined	Spleen-rate	Crescent-rate	Number examined	Spleen-rate	Crescent-rate
Naras (Sumatra)	Schüffner, 1919 <i>b</i>	307	4	1.3	13	.	.	65	1	410	84	40	290	70	36
Angoli (Sumatra)	"	96	7	7.3	6
Western Java	Mangkoe Winoto, 1918	9
Batavia (Java)	v. Breemen, 1918-19
Siantar (Sumatra)	Bais, 1919
Sigiran (Sumatra)	Swellengrebel, 1918 <i>a</i>
Belawan (Sumatra)	Swellengrebel and Schüffner, 1919	1106	.	.	6	3	114	87	13	709	96	0.9
Mandailing (Sumatra)	<i>id.</i>	1056	96	9	.	.	.
Kepetakan (Java)	<i>id.</i>	8	209	76	40	.	.	.
Soerabaia (Java)	<i>id.</i>	79	.	.	28	.	.	31	653	84-100	3-17	.	.	.
Soendatar (Sumatra)	<i>id.</i>	298	90	48	320	87	34
Tegal (Java)	Swellengrebel, 1919 <i>c</i>	41	.	.	8	391	91	15	354	91	1.7
Semarang (Java)	"	129	3	2.3	262	80	10	601	74	2.7
Modjowarno (Java)	1918 <i>d</i>	197	37	10	184	32	13
Soerabaia (Java)	1919 <i>d</i>
Mandailing (Sumatra)	Reylingh, 1918
	Schüffner, 1916

Fed. Malay States	Stanton, 1914	78	3	3.8	32	2	6.2	20	6
<i>id.</i>	Barber, 1918	66	.	.	11	1	9.1	60	261	6	2.3
<i>id.</i>	Watson, 1911	.	.	.	19	4	21.0	10	1	10.0
<i>id.</i>	Strickland, 1916	38
Brit. N. Borneo	Roper, 1914	17	.	.	.	130	1	0.8
Grand total		144	3	2.1	62	7	11.3	80	.	.	17	.	.	445	8	1.8

N.B. The structure of the pigment of the Anophelines found infected in the Malay Archipelago was as follows:

	Fine pigment recorded		Coarse pigment recorded	
<i>ludlowi</i>	176 times	...	188 times
<i>rossii</i>	11 "	...	7 "
<i>leucosphyræ</i>	2 "	...	2 "
<i>barbistrois</i>	1 "	...	2 "
<i>indefinita</i>	2 "	...	1 "
<i>sinensis</i>	2 "	...	5 "
<i>fuliginosus</i>	1 "	...	1 "
<i>aconita</i>	1 "	...	2 "
<i>punctulata</i>	1 "	...	—

that *M. barbirostris* is comparatively negligible as a cause of severe endemic or epidemic malaria.

This method of comparison, in neighbouring regions, of the parasitaemia and of the spleen-rate of the inhabitants, with the Anopheline fauna, enables us to ascertain the presence or absence of a correlation between the occurrence of a severe malaria and that of a particular species of Anopheline, and leads us to the determination of the *indirect index of infectivity* of this species, or shortly "indirect index" (I. I.). Taken alone, conclusions drawn from the I. I. are open to adverse criticism, but if the N. I. is likewise known, the conjoint data will furnish information as to the actual amount of damage done by the species.

Taking into consideration the figures given in Tables III and IV, together with the determinations of the I. I. for each species, we may summarize the facts as follows:

1. **M. ludlowi** is the most important vector of malaria in the Malay Archipelago, and shows the highest degree of N. I.; its importance is enhanced by the following propensities: the adult shows a preference for the interior of human dwellings; it usually occurs in large numbers; and it is able to cover by flight considerable distances. We have observed (1918 c) that large numbers of adults in nature habitually cover distances of 1.6 km., as measured in the direct line from the place of capture to the nearest breeding-place. We excluded all instances in which there was not absolute certainty of the absence of nearer breeding-places, and, moreover, all cases were excluded where only a few Anophelines could be found in the place of capture. Some small breeding-places might after all have been overlooked, and a few Anophelines might have been derived from this hypothetical breeding-place, but a number amounting to several hundreds could not be accounted for in this way. The majority were certainly derived from the extensive breeding-places under observation.

In two cases we succeeded in infecting Europeans with subtertian, by exposing them to the bites of *M. ludlowi*, which had been fed on a crescent-carrier twelve days previously. Ourselves being the subjects of this experiment, we are assured of the absence of any previous malaria infection. Moreover, the experiment was performed in a locality some 12 km. from the coast, where *M. ludlowi* was absent and malaria infection rare.

With few exceptions we found severe malaria to prevail wherever *M. ludlowi* was present.

2. **M. aconita** (including *M. minima*). Some doubt exists as to the ability of this species to transmit malaria. Winoto (1918) found it to possess the highest N. I. (7 per cent.) of all the species in very malarious hill-districts. We found it (1919 b) to be the cause of a rather severe epidemic in a previously non-malarious district, and exhibiting an N. I. of 2 per cent. In the highly malarious regions of Mandailing and Soendatar, however, we failed to

find infected females of *aconita*, only reputedly bad vectors, like *sinensis*, *barbistrois*, *indefinita*, and *fuliginosus*, were found infected. *M. aconita* was often found in districts where the incidence of malaria was insignificant. Attempts at experimental infection rarely succeeded (Schüffner). It seems therefore, that the infectivity of this species is subject to considerable variation, and its importance depends, to a large extent, on its occurrence in large numbers and throughout the year. If present in scanty numbers, or within the limits of particular seasons, it may cause epidemics of greater or less severity, but it does not seem to be capable of permanently raising the splenic index distinctly, as is the case with *M. ludlowi*, notwithstanding that the latter species shows a marked seasonal prevalence, and may be absent for a whole year. Although the importance of *M. aconita* should not be underrated, it is negligible in comparison with *M. ludlowi*.

3. **N. maculatus.** Infected specimens were never found, though sought for in highly malarious regions, where *M. sinensis* and *N. fuliginosus* showed infection (Soendatar), but the numbers examined were too small to allow of any definite conclusions being reached. On the other hand, the indirect index often points it out as a dangerous species, for its absence or presence in neighbouring districts, which otherwise possess a similar Anopheline fauna, coincides with the absence or presence of severe malaria. There are exceptions to this rule, however: in Siboga *N. maculatus* prevailed in the hills, while malaria was mainly confined to the seashore (*M. ludlowi*).

Here again, as with *M. aconita*, we must infer a high degree of variability in the power to rear malaria parasites on the part of the vector.

4. **N. leucosphya.** Schüffner and Swellengrebel (1914) observed a distinct correlation between the appearance of this species and local outbreaks of malaria on tobacco estates and in certain coolie lines. Similar observations have been recorded by Roper (1914). Recently, Bais (1919) has confirmed and extended these observations by finding infected specimens (N. I. = 1.7 per cent.). He also succeeded in attempts at experimental infection with tertian parasites, the rate of infection, however, not surpassing that of *M. sinensis* as observed by us in some of our experiments (50 per cent.).

5. **M. sinensis** and **N. fuliginosus.** Both of these species were found infected in malarious regions, the former commonly so, but the N. I. was uniformly low (under 1 per cent.). Experimental infection of *M. sinensis* with tertian succeeded to a fairly high degree (at times over 50 per cent.); infections with subtertian and quartan were rarely successful. Experimental infection in man (with the same precautions as those taken with *M. ludlowi*) succeeded twice. *N. fuliginosus* was infected experimentally by Schüffner with subtertian (4 per cent.). The two species are common in both malarious and salubrious regions; in the former case obviously infected carriers are frequent. But in Soendatar, a region of severe malaria, we found them

to be the only Anophelines infected, all others, including *M. aconita* and *N. maculatus*, failing to show malaria parasites. In this case we must admit one or both of these species to be a possible vector, although the indirect index pointed to *N. maculatus* as being the principal vector.

6. **M. umbrosus.** This species was found infected but once. Experimental infection succeeded with tertian only, at a rate much below that of *M. sinensis*. Therefore *M. umbrosus* cannot be admitted as the cause of a severe epidemic, moreover, the indirect index never pointed to its being a dangerous species; still we mention it separately, because Watson (1911) considers it to be the chief vector in the plains of the Malay Peninsula, and Barber (1918) succeeded in infecting specimens from some localities and not from others. We presume therefore that its infectivity is variable, and it should, at least, be suspected. Nevertheless, *M. umbrosus* has not the importance which attaches to *M. aconita*, *N. maculatus* and *M. ludlowi*.

7. In this section we shall consider certain species with a relatively low N. I. (under 1 per cent.), although in epidemic regions the N. I. may rise to 3 per cent. (*M. rossii*). The species in question are *M. rossii*, *M. barbirostris*, *N. punctulata* (*tesselata*), and *M. indefinita*. These species are not confined to malarious regions. We are inclined to include *C. kochii* in this category although Schüffner (1918 b) once found it to be more highly infected than the presumed local vector (*M. aconita*).

All of these species have been infected experimentally, but it would appear as if natural infection of the vectors is small in regions of low endemicity owing to the paucity of gamete-carriers. Of the species enumerated, *M. indefinita* is the least important.

8. **S. aitkenii** (with its larval varieties *insulae florum* and *papuae*). Further information regarding this species is required. It was found exclusively in highly malarious regions associated either with *M. aconita*, *N. maculatus*, or *N. annulipes* var. *moluccensis*. The last species is the most common Anopheline in the eastern part of the Malay Archipelago; regarding its infectivity we can say nothing for the present. Furthermore we have failed to find natural infection in *M. flava*, *M. albotaeniatus*, *M. mauritanus*, *M. gigas*, *N. schüffnerii*, *N. jamesii*, and *N. kawarii*.

Arranging the above species with regard to their epidemiological significance, we obtain the following sequence:

(1) *Myzomyia ludlowi*; (2) *Myzomyia aconita*, *Nyssorhynchus maculatus*, (perhaps also *Stegomyia aitkenii*); (3) *Neomyzomyia leucosphyra*, *Myzorhynchus umbrosus*; (4) *Nyssorhynchus fuliginosus*, *Myzorhynchus sinensis* (? = No. 6); (5) *Cellia kochii*; (6) *Myzomyia rossii*, *Myzorhynchus barbirostris*, *Neomyzomyia punctulata* (*tesselata*); (7) *Myzomyia indefinita*.

II. Habitats of the Anopheline Larvae.

One of the conditions needed for the institution of specific sanitary measures, appears to hold in the Malay Archipelago. In all highly malarious districts studied hitherto, only one or two species are certainly or presumably principal vectors, and the remainder may be neglected. But are we justified in neglecting them? Is it possible to carry out sanitary works, directed against these known vectors, without including all other Anophelines in that locality? In other words, are the breeding-places of known vectors sufficiently differentiated to allow of "specific sanitation" (see p. 181)? This problem we must solve, otherwise our measures may prove useless.

A species may be particular in the choice of its breeding-place, or on the other hand it may show a considerable degree of adaptability in this respect. When observations are conducted in but one locality, it is often impossible to form a correct opinion on this subject, even after prolonged investigation. In the course of observations lasting nearly a year, we found (in Modjowarno, East Java) the larvae of *M. aconita* inhabiting rice-fields exclusively, and usually fields about to be harvested. In other localities this species was found to be more generally distributed (see Table V, p. 194).

The larvae of *N. maculatus* were found only in hilly districts; they are generally confined to little streams and springs on hill slopes. Consequently it might be supposed that sub-soil drainage, etc. would eliminate the larva. Unfortunately, we have noted abundant larvae of this species in a variety of waters (rice-fields, puddles, brackish pools near the seashore, cement tanks, etc.) during the dry season when the small streams above alluded to are dried up (see Table V).

The larvae of *M. umbrosus* occur in fresh and brackish water near the seashore and elsewhere, showing a preference for shade, but sometimes occurring in the open with no other protection against the sun than that afforded by short rushes. The larvae of *N. leucosphyra* on the other hand invariably affect shady pools, without discrimination in other particulars, the water being clean or muddy, stagnant or running. Our observations regarding the latter species are few (see Table V).

The larvae of most of the other species occur in a variety of waters. Some, like *M. aconita*, select one particular type, almost to exclusion of others; in Mandailing for instance, we found larvae of *M. barbirostris* frequenting fish-ponds, but comparatively rare in the rice-fields, where *M. sinensis* was the commonest larva. In other localities, however, *M. barbirostris* larvae were even more numerous in the rice-fields than were those of *M. sinensis*. This observation also applies to other species which are very particular in their choice of a breeding-place; in other districts they appear to be independent of special breeding grounds (see Table V).

A few species never leave us in doubt as to their dependence on particular breeding-places; some species are ubiquitous—*N. annulipes* var. *moluccensis*

may be found breeding in all imaginable collections of water (salt, brackish, fresh, dirty, clean, running or stagnant). In the absence of natural breeding sites, it will thrive in artificial ones (water in boats drawn up on shore, water in coconut shells, etc.) as readily as will *Culex fatigans* or *Stegomyia scutellaris*. The larva of *M. indefinita* behaves in a similar way, except that it is not often found in distinctly brackish water: we even found it breeding in saucers, of water, placed to prevent the access of ants. *C. kochii* and *N. punctulata* (*tesselata*), though somewhat more fastidious, distinctly belong to this group.

Still there are limits to the breeding possibilities of these species. In water, highly polluted with faecal matter, the larvae are absent; we once found *N. punctulata* in this situation (see Table V). Two species were found to be very limited in the choice of a breeding-place. The first of these, *S. aitkenii*, occurred in swiftly running mountain streams in 97 per cent. of cases. In this restriction of habitat it compares with what is stated concerning *N. maculatus*. If *S. aitkenii* proves to be a vector of malaria, this knowledge may be put to practical use. The second species, *M. ludlowi*, has at present a more practical importance. It is found in two morphologically indistinguishable varieties, which, on physiological grounds, must be dealt with separately. The first or *littoral form*, is almost confined to salt or brackish water along the coast, especially in places where algae (*Enteromorpha*, *Cladophora*, *Cyanophyceae*) and other filamentous aquatic plants, like *Najas*, are growing. In such breeding-places, during the dry season, June–September, the larva may be found in immense numbers. It is occasionally found in fresh water, but even then it is limited to the coastal zone, rarely penetrating inland for more than 4 km., except in such places where brackish water is to be found far inland (tidal rivers). Where the tide runs in and out freely, as in mangrove swamps, it appears to be absent; but if human intervention, by the construction of bunds, locks, etc., creates obstacles to the tidal water, the conditions change; grass begins to grow between the roots of the rhizophores, and larvae of *M. ludlowi* soon begin to appear. A place comparatively healthy before the commencement of the operation is thus rendered highly malarious (see Table V).

The larvae of the *inland form* of *M. ludlowi* are to be found only in the narrow valleys among the mountains running parallel to the west coast of Sumatra, where they occur in numerous fish-ponds. These ponds are almost the only breeding-places of this species, but it may breed in rice-fields also, though rarely. In one of the valleys, an extensive lake (Manindjau) is situated, along the banks of which this form is common among the rushes and algae, a frequent habitat of many Anopheline larvae (Table V).

Table V. Showing the number and habitats of the several species of Anophelines together with the percentage frequency of each species in various breeding-places.

Type of breeding-place	M. ludlowi littoral form		M. rossii		M. ludlowi inland form		N. leucosphysa		M. umbrosus		M. indefnita		C. kochii		N. punctulata		M. sinensis	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
I. NATURAL BREEDING-PLACES.																		
A. In salt or brackish water.																		
1. In fishponds ...	1282	54.5	2028	30.3
2. Elsewhere ...	814	34.6	4339	64.9	141	51.1	103	2.2	6	1.9	26	3.3	58	0.6
B. In fresh water.																		
3. In immediate neighbourhood of sea coast ...	233	9.9	223	3.3	.	.	25	8.3	63	22.9	260	5.5	43	13.7	49	6.3	19	0.2
4. In rice-fields, in plains and hills ...	4	0.16	18	0.3	12	0.4	1291	27.4	34	10.9	272	35.0	4748	50.2
5. In little streams (running), their springs, marshes formed in their course, irrigation ditches for rice-fields ...	4	0.16	7	0.17	.	.	61	20.2	60	22.0	1006	21.3	43	13.7	102	13.1	1163	12.3
6. Marshes and pools, containing clear water, much overgrown ...	2	0.08	46	0.69	4	1.6	342	7.2	20	6.4	65	8.3	200	2.1
7. Fish-ponds (fresh water)	6022	97.9	808	17.1	66	21.4	47	6.0	2990	30.5
8. Pools in jungle...	216	71.5	2	0.8	.	.	26	8.3	135	17.3	.	.
9. Pools and small puddles with dirty water	899	19.1	75	23.7	82	10.7	283	3.0
10. Great inland lakes (along the shore)	108	1.7	3	0.08
II. ARTIFICIAL BREEDING-PLACES.																		
11. Cemented tanks, water in native boats, in coconut shells, earthenware saucers, etc.	14	0.6	23	0.34	4	1.6	4	0.12	1	0.01
Grand total...	2353	100	6684	100	6142	100	302	100	274	100	4716	100	313	100	778	100	9462	100

Table V—continued.

Type of breeding-place		<i>N. fuliginosus</i>		<i>M. barbirostris</i>		<i>S. aitkenii</i>		<i>M. aconita</i>		<i>N. maculatus</i>		<i>N. karuvarii</i>		<i>M. albotaeniatas</i>		<i>N. annulipes</i> var. <i>moluccensis</i>	
		Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
I. NATURAL BREEDING-PLACES.																	
A. In salt or brackish water.																	
1. In fishponds
2. Elsewhere
B. In fresh water.																	
3. In immediate neighbourhood of sea coast	...	31	1.9	90	1.0
4. In rice-fields, in plains and hills	...	262	16.1	929	10.2	3	1.9	782	45.0	46	8.0	135	22.8
5. In little streams (running), their springs, marshes formed in their course, irrigation ditches for rice-fields	...	375	23.1	1051	11.5	152	97.4	567	32.7	246	42.9	26	100	.	.	63	10.6
6. Marshes and pools, containing clear water, much overgrown	...	87	5.3	1326	14.5	.	.	119	6.8	189	33.0	115	19.4
7. Fish-ponds (fresh water)	...	844	51.9	5431	59.6	.	.	261	15.0	23	4.0
8. Pools in jungle...	36	0.4	37	100	15	2.5
9. Pools and small puddles with dirty water	...	18	1.1	175	1.9	.	.	7	0.5	34	6.0	36	6.1
10. Great inland lakes (along the shore)	...	2	0.2	3	0.07
II. ARTIFICIAL BREEDING-PLACES.																	
11. Cemented tanks, water in native boats, in coconut shells, earthenware saucers, etc.	...	1	0.1	9	0.23	34	5.9	54	9.2
Grand total	...	1625	100	9110	100	156	100	1736	100	573	100	26	100	37	100	592	100

III. Practical importance of the larval habitat.

It is evident that this study possesses a more than academic importance. Let us imagine the effect which would be caused by an apparently unimportant change in the habitat of such a species as *M. ludlowi*, which we may suppose to acquire the breeding habits of *M. indefinita*. Such an occurrence in the Malay Archipelago would spell disaster. The severe endemic malaria, at present confined to the sea coast, and strictly localized in the interior, would lead to the spread of the disease over wide areas in a manner corresponding to what is seen in some parts of Africa. All this would result from an exchange of habitat between two Anophelines, so closely allied morphologically that some entomologists refuse to consider them as distinct species. Moreover, the eradication of the larvae would become a practical impossibility; for an Anopheline capable of breeding in all possible collections of water, in a country of high humidity and heavy rainfall, and where the population is dependent on the cultivation of rice, cannot be abolished.

Although conditions in the Malay Archipelago are not so bad as this, still the prospects are not uniformly favourable, even if our purpose aims, not at the complete suppression of malaria, but only at checking the more severe endemic and epidemic manifestations. We have in that case to exterminate only *M. ludlowi*, *M. aconita*, *N. maculatus*, perhaps *S. aitkenii*, and in some places perhaps *N. leucosphyra* and *M. umbrosus*. It will simplify our task considerably, provided these species are restricted in their breeding-places, otherwise we may as well commence our operations against all local Anophelines, excepting only the breeding-places peculiar to *M. indefinita*, *C. kochii* and *N. punctulata* (*tesselata*).

This, unfortunately, is the attitude which we have to take with regard to *M. aconita* and *N. maculatus*. For reasons mentioned above, specific sanitation cannot be put into operation. Even if in a certain district, one of these species seems to be limited to one type of breeding-place, still we cannot advise the exclusive abolition of the larva, because observations from other localities have taught us that it may readily take to another kind of breeding ground. Nevertheless the study of the breeding habits of these mosquitoes has given us some useful knowledge. We have often observed careless cultivation of the rice-fields by the natives; the fields are inundated before being sown, in order to simplify the operation of ploughing; after harvesting, the water is not allowed to drain off, and, in consequence, the rice-fields are converted into swamps. Fields are frequently seen, in which the rice-stalks lie down in the water, because the owner of the field neglected to cut the ears in time. Such rice-fields constitute favourable breeding places for many species of Anophelines, among them *M. aconita* and *N. maculatus*. This negligence is harmful, not only from the sanitary standpoint, but also for agricultural reasons, and the latter reason alone should help to bring about an improvement.

On the other hand, whenever the species occurs in well-cultivated rice-fields, nothing at present can be done to prevent its propagation.

The larval habitat of the "littoral" *M. ludlowi*, on the contrary, facilitates sanitary measures; also, the "inland" form may be exterminated by the abolition of fish-ponds. Without improvement in the methods of rice cultivation, however, little can be accomplished.

CONCLUSIONS.

1. The eradication of Anopheline larvae, in the Malay Archipelago, is difficult because they breed in all kinds of temporary collections of water, which constantly reappear throughout the rainy season.

2. Anti-larval measures directed against *M. aconita* and *N. maculatus* are hard to carry out; specific sanitation is rendered difficult by their breeding habits, but amelioration may be expected as the result of improved methods of rice cultivation.

3. Specific sanitation is practicable in malarious regions where *M. ludlowi* is the chief vector.

The observations detailed in this paper might lead to the supposition that, in the Malay Archipelago, our investigations have been more academic than practical. This is not so. In several places on the sea shore (Siboga, Belawan in Sumatra, Batavia, Tegal, Semarang, Soerabaia, Tjilatjap in Java), and in some localities of minor importance in the interior, anti-malarial operations are in progress, or in preparation, but years must elapse before we can appreciate their effect. Where a distinct amelioration has been observed (e.g. Siboga) we are uncertain as to whether this should not be attributed to a remission in the intensity of endemic malaria, so frequently observed in the Malay Archipelago. So soon as conclusions may be drawn regarding the efficacy of our sanitary measures, we hope to make them known.

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ON THE LIFE HISTORY OF *BUCENTES* (*SIPHONA*)
GENICULATA (DIPTERA: TACHINIDAE), PARASITE OF
TIPULA PALUDOSA (DIPTERA) AND OTHER SPECIES¹.

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(With Plate XIV.)

THE Dipterous Family, Tachinidae, comprises a very large number of species whose larvae live as parasites within other insects, particularly in their larval forms.

Bucentes (*Siphona*) *geniculata*, one of the Tachinidae whose larvae are considered in the following paper, is a small ordinary looking fly, blackish in colour and showing a somewhat greyish abdomen, with prominent abdominal bristles. The labium is long and slender, sharply geniculated about the middle of its length, and folded like a clasp knife under the head when not in use².

In a former paper (1912) one of us has recorded the occurrence of the larva of this species as a parasite in the body cavity of *Tipula* larvae. It is probably not confined to one host species. In one collection of *Tipula* larvae, a proportion of which yielded the parasite, the majority of the survivors, on hatching, proved to be *Tipula oleracea*. In most instances, however, we have found the infected insects to be *T. paludosa*.

Since the original observation in 1912, we have found this larva regularly every year, and consequently regard it as a normal parasite of *Tipula*. Other observers record it from *Mamestra brassicae* and a related species, *Siphona cristata*, is reported by Roubaud (1906) to occur in *Tipula gigantea*.

OCCURRENCE AND DISTRIBUTION OF THE ADULT FLY.

From an examination of records relating to the adult insect, kindly submitted to us by the entomologists in charge at the British Museum and the Royal Scottish Museum, we find the fly is widely distributed throughout

¹ This work has been carried out with the aid of Grants from the Board of Agriculture for Scotland, to whom we desire to express our thanks.

² A full description of the structure and habits of the adult fly is reserved for a further paper

Great Britain. It is recorded from Devon, Hereford, Hants, Kent, Radnor, Middlesex, Hampshire and Inverness-shire. We have found it in Northumberland and Aberdeenshire.

The adults of this fly are recorded over the period from May till September. Schiner (1862) writes regarding the genus: "The flies live in dry places especially heaths. They are also found on Umbelliferae and on waste ground are often to be seen in large crowds on *Daucus carota*." He also states that *B. geniculata* visits *Erica vulgaris*. Walker (1853) reports these flies as "very common."

The following list of flowers known to be visited by *B. geniculata* is compiled from Knuth's *Handbook of Flower Pollination* (1909).

<i>Viola lutea.</i>	<i>Valerianella olitoria.</i>
<i>Stellaria holostea.</i>	<i>Succisa pratensis.</i>
<i>Medicago lupulina.</i>	<i>Eupatoria cinnabinum.</i>
<i>Potentilla sylvestris.</i>	<i>Pulicaria dysenterica.</i>
<i>Potentilla sterilis.</i>	<i>Myosotis sylvatica.</i>
<i>Daucus carota.</i>	<i>Mentha aquatica.</i>
<i>Hedera helix.</i>	<i>Origanum vulgare.</i>
<i>Asperula cynanchica.</i>	<i>Hottonia palustris.</i>
<i>Asperula odorata.</i>	

METHODS OF INVESTIGATION.

The *Tipula* larvae, after being washed free of soil, were examined individually and with a little practice the parasitic maggot could be readily recognised beneath the skin of the host larva as an elongated yellowish patch of definite shape.

The proportions found to be infected varied a good deal in different localities and in different years. In one area, viz. Echt, Aberdeenshire, the numbers reached a significantly high figure. Over a period of one month, during which larvae were examined, a progressive decrease in the percentage of infected individuals was noted. This was found to be due to the fact that as the season advanced, the infected larvae were dying off, and pupae of *Bucentes* were becoming increasingly more numerous in the soil. It must therefore be borne in mind that for the first generation, unless examinations are made not later than about February, any estimate of the degree of infection must be below that actually existing. The following data illustrate this:

LARVAE COLLECTED IN ECHT AND LOWER DEESIDE AREAS.

Data for 1918.

Lot	Date	Infected larvae	Healthy larvae	Total	Percentage infected
1.	Feb. 20	170	434	604	28.1
2.	„ 27	127	243	370	34.3
3.	„ 28	164	524	688	23.8
4.	Mar. 1	73	386	459	16.0
5.	„ 6	103	442	545	19.0
6.	„ 7	182	819	1001	18.2
7.	„ 11	56	345	401	13.7
8.	„ 16	42	258	300	14.0
9.	„ 23	79	221	300	26.3
Totals		996	3672	4668	21.3

LARVAE COLLECTED IN NORTHUMBERLAND.

For purposes of comparison we obtained a lot of larvae from the north of England. These yielded the following result:

Date	Infected larvae	Healthy larvae	Total	Percentage infected
Mar. 1	51	794	845	6.0

In other areas where larvae were collected in fewer numbers, the parasite was also found and all our observations suggest that it is relatively abundant.

During the season 1919, *Tipula* larvae were again collected and further data concerning the occurrence of *Bucentes* parasites were obtained.

Search was made for *Tipula* larvae in fields on the College of Agriculture experimental farm at Craibstone, Aberdeenshire, from the month of December onwards. During December and January only very small numbers of *Tipula* larvae were found and of these none were infected with *Bucentes*. In February and March the numbers found increased slightly and out of 56 larvae, three were found infected, one of these harbouring four parasitic maggots.

From April onwards appreciable numbers were obtained; the data for the season are shown in the table given below:

LARVAE COLLECTED IN LOWER DEESIDE AND LOWER DONSDIE.

Data for 1919.

Lot	Date	Infected larvae	Healthy larvae	Total	Percentage infected
<i>Craibstone.</i>					
1.	April 7	25	349	374	6.6
2.	„ 11	39	237	276	14.1
3.	„ 22	56	429	485	11.5
4.	„ 25	46	288	334	13.4
5.	May 8	1	155	156	0.6

No infected larvae obtained here during period May 9 to June 20.

LARVAE COLLECTED IN LOWER DEESIDE AND LOWER DONSDIE (*continued*).*Data for 1919.*

Lot	Date	Infected larvae	Healthy larvae	Total	Percentage infected
<i>Craibstone.</i>					
6.	June 21	13	66	79	16.45
7.	„ 25	23 (+7 otherwise infected)	30	60	38.6
8.	„ 30	22 (+6 otherwise infected)	49	77	28.5
9.	July 7	6	9	15	40.0
<i>Angusston, Culter.</i>					
10.	April 26	1158	3467	4625	25.0
11.	May 2	107	456	563	19.0
12.	„ 9	50	903	953	5.2
13.	„ 16	3	807	810	0.37
		<hr/> 1562	<hr/> 7245	<hr/> 8807	<hr/> 17.6

As is indicated in the foregoing table no infected larvae were obtained between May 16th and June 21st. This was the case in other areas in the north of Scotland of which several were examined.

The fall to 0.6 per cent. at the beginning of May, and the complete absence of infected larvae in the soil for nearly five weeks, we attribute to the fact that the *Bucentes* maggots had pupated, and thus the *Tipula* larvae then found were such as had escaped infection by the parasite.

Larvae obtained at Craibstone on June 21st showed an appreciable percentage infected by *Bucentes* larvae, and from that date until July 7th, when the oats had grown too high to allow of further search for grubs, infected larvae continued to be obtained in high percentages, as is shown in the table.

NUMBER OF PARASITIC LARVAE PER HOST.

Observations made in 1918 show that one, two, three, and even four parasitic maggots may occur in one host. This was also the case in specimens found in the early months of 1919. A noticeable feature among the larvae found in the later part of 1919, viz. from June 21st onwards, was that there were seldom fewer than two *Bucentes* maggots in each host larva, 5-6 being common, and in one case as many as nine *Bucentes* maggots were found in a single host.

SIZE OF PARASITIC LARVAE FOUND.

Larvae from 1.5 mm. up to 8.5 mm., which represents the maximum size, have been found.

Even within the same host, the larvae may differ in size. For example, two maggots, 5 mm. in length, were found in the same host along with two others of 3 mm. In one case, where nine parasites occurred together, these were all 3 mm. in length. It seems more probable to us that these differences

in size in parasites occurring together are due to variations in the rate of growth than that the same host should have been parasitised on two or more separate occasions.

DURATION OF LARVAL PERIOD.

First Generation. In the season 1918, *Bucentes* larvae were obtained as early as February 20th. These larvae were on an average about 4 or 5 mm. in length at this date. In 1919, these larvae were first obtained on February 28th; they were from 3 to 5 mm. in length. This suggests that the parasitic larvae found in February are the product of eggs laid the previous autumn, the larvae having hibernated in the host.

Second Generation. From June 21st onwards, large percentages of the collected host larvae were found to be infected with *Bucentes*, whose maggots were of such a size, viz. 1–3 mm., as to lead to the suspicion that the *Tipula* larvae had been recently infected. From these *Bucentes* maggots a second generation of flies was bred which began to emerge on July 25th. An earlier generation of flies was bred out in May and the early part of June and by June 13th all these flies had died. Moreover, from May 16th to June 16th *Tipula* larvae were collected on four different occasions, to the number of 355, and of these none were found to be infected by *Bucentes*. This evidently indicates that all the parasites had pupated, involving the death of the host larvae, so that the *Tipula* larvae collected between these dates were those which had escaped infection by *Bucentes* during the previous autumn. In view of the foregoing, viz. that there is a period of over four weeks during which no infected *Tipula* larvae can be found in the field, that within this period adult flies appear to die off and that subsequently infected larvae again appear, the conclusion seems obvious that a second generation of *B. geniculata* commences about this time.

Bucentes maggots of the second generation were observed on June 16th, when they measured about 3 mm. The earliest date on which mating was observed was June 2nd, and no imagines survived in the observation cage after June 13th. Oviposition, and the infection of *Tipula* larvae in the field, may therefore be assumed to take place approximately within a corresponding number of days. Pupation of the second generation of larvae was observed to begin in the experimental cage about July 8th. That is to say, the maximum extent of the larval period possible was in this case from June 3rd to July 8th, viz. five weeks. This estimate is only an approximate one, as oviposition has not been observed and the period which elapses between oviposition and the hatching of the larva is unknown.

We have been able to find in the literature dealing with this subject only the scantiest references to this species. Nielsen (1918), in a table showing the life cycle of a number of Tachinidae, includes *Bucentes geniculata* in the form reproduced below, wherein P. signifies Pupa, L. = Larva, I. = Imago.

	April—May	June	July	Aug.	Sept.	Oct.	Nov.—April
<i>Bucentes geniculata</i>	P.I.	L.P.I.	I.	I.L.	I.L.	P.	P.

Unfortunately, there is no evidence in the paper regarding the locality to which the data relate, but it would appear that the species may exist in the pupal condition from October to April.

For comparative purposes we append our data, which show that we are familiar with the life cycle of the parasite during the period from February to August, and that our information would indicate that the winter is spent in the larval condition in our area. Both records agree in showing that two generations occur in the year—a short-lived summer and a longer winter generation.

	April—May	June	July	Aug.	Sept.	Oct.—Nov.	Feb.	March	April
<i>Bucentes geniculata</i>	P.I.	I.L.	L.P.I.	I.	?	?	?L.	L.	L.P.

PUPATION.

Observations show that usually the maggot leaves the body of its host and pupation takes place outside in the soil. After having emerged from the host larva, the parasitic maggot may still remain attached, as described later, to the tracheal trunk of the host, but usually the separation from the host, preparatory to pupation, is complete. In one instance, however, pupation was observed to have taken place within the host larva—the latter being still alive.

Within twenty-four hours after the maggot leaves its host, pupation is complete.

PERIOD OF PUPATION.

First generation. In the season 1918, pupation was first observed on April 1st. The earliest date on which *Bucentes* flies emerged was April 23rd, so that approximately pupation extends over a period of three weeks.

In the season 1919 puparia were first observed on April 24th and flies began to emerge on May 14th. In this instance, the duration of pupation was 20 days.

Second generation. From observations made on pupae obtained from larvae of the second generation in the season 1919, we found pupation to extend from July 8th to 25th, *i.e.* over a period of 17 days.

EMERGENCE OF ADULTS.

In 1918 adult *Bucentes* flies were seen in the observation cage as early as April 23rd. Emergence continued until June 4th, after which date all the flies had died off.

In 1919, when the weather was less favourable, no flies were seen until May 14th. By June 13th all the flies had died.

In 1918, emergence continued therefore over a period of about six weeks. The flies which issued in April were rather small, but those which emerged during May were normal in size.

In 1919, emergence of flies continued only for about four weeks. In the

case of the second generation which began to appear on July 25th, the period during which emergence took place was about three weeks.

In order to find out from what depths the adult fly could emerge, the following experiments were set up.

Glass cylinders were filled with earth; in one the soil was loose, in another the soil was moderately compact. Puparia were placed at various depths, these being indicated by strips of gummed paper at the same level on the glass cylinder.

It was found that in loose soil the adult fly emerged from puparia placed at a depth of 2 and 3 inches, but did not emerge from puparia placed 6 inches below the surface. In moderately compact soil, the adult fly emerged from puparia placed at a depth of 3 inches, but not from a depth of 6 inches. The puparium cases were afterwards found at the levels at which they were originally placed. It has been noted that the parasitic maggot, after leaving the dead host, is capable of moving about in the soil, and probably it moves upward near the surface before pupating.

MATING.

Mating took place freely in the cage; in 1918 from May 16th to the end of the month; in 1919, from June 2nd till about the 12th.

Mating *Bucentes* were isolated in a test-tube with one or two daisy blossoms and kept under observation. The flies were found to remain *in coitu* over a period of about two hours. Oviposition was not observed, but flies which had mated were found to survive 4-5 days.

During both seasons, 1918 and 1919, pairs of mating flies were isolated along with *Tipula* larvae, and also in view of their recorded occurrence in *Mamestra*, along with various caterpillars, e.g. *Agrotis exclamationis*. No infection took place under these conditions.

LONGEVITY OF THE IMAGO.

Experiments were carried out to ascertain longevity of adult *Bucentes*, wherein newly-emerged flies were isolated in glass cylinders. In the bottom of the cylinder was put about 2 inches of earth in which one or two flowering daisy plants were placed to provide food for the flies. Under these conditions the flies survived 5 to 10 days. Under natural conditions they doubtless live longer than 10 days.

The confined flies are strongly attracted to light, they are exceedingly active and run about freely upon the surface of the soil. This was planted with daisies and dandelions, which were much sought, the flies being seen to introduce their proboscides into the daisy florets and to feed on the pollen.

SUMMARY OF LIFE HISTORY.

So far as we have been able to trace the life history of *Bucentes geniculata* it may be summarised as follows:

The winter months are spent as larvae within their hosts, viz. *Tipula* larvae. Pupation may start as early as the beginning of April if the season is good but in a late season, pupation may not begin until nearly the end of this month. After a pupal period of about three weeks the imagines emerge during April and May. By the middle of June the adult *Bucentes* are dying off. A second generation appears in June. After a larval period of about three weeks and a pupal period of about seventeen days the adult flies emerge towards the end of July. Since *Tipula* larvae are found in the winter months parasitised with *Bucentes*, infection probably takes place in the autumn whilst the *Tipula* larvae are comparatively young¹.

STRUCTURAL RELATIONS OF THE PARASITIC LARVA TO ITS HOST.

Dufour (1827) described the larva of the Tachinid *Ocyptera bicolor*, which inhabits the body cavity of an Hemipteron, *Pentatoma grisea* Latr. He observed a membranous funnel-like structure, equal to about one-third of the length of the larva, extending from the last segment of the body and adhering by means of a pair of horny teeth at the other end to a metathoracic stigma of its host. He noted that this "siphon" could be detached from the larva without injuring it and he also found in other cases, "siphons" adherent to the metathoracic stigmata of the *Pentatoma* in the absence of the parasitic larvae.

Kunkel d'Herculais (1879) described a similar structure in the larva of *Gymnosoma rotundatum* Linn., and regarded the siphon as a product of the larva. In a Tachinid larva of *Carabus*, Cholodkowsky (1884) found a similar structure fixed to a trachea and described it as a chitinous funnel. He interpreted this as a pathological chitinous product produced by the hypodermal layer of the trachea at the point where the larva, having entered by a stigma, perforated the tracheal system to reach the body cavity.

Pantel (1898 and 1909), in *Thrixion Halidayanum*, clearly established the true nature of this funnel as an inflammatory reaction on the part of the host, and has more particularly shown how these anatomical relations are established between the host and parasite by a development of the hypodermal cells of the skin or of the trachea, whichever structure was utilised by the larva as a means of bringing its stigmata into direct relation with the external air. The perforation of the host's tissue at the point where attachment eventually takes place is effected primarily by means of the hooklets or spines which surround the stigmatic area of the parasite.

Roubaud (1906) describes the relations between *Siphona cristata* and its host, *Tipula gigantea*, in the following terms:

"A ce stade relativement jeune, chaque parasite est encore complètement inclus dans un kyste fermé, membraneux, fixé au cordon trachéen par une sorte de calice chitineux dont le fond s'ouvre dans la trachée chez les larves

¹ The summary of the life history as given by Nielsen, is quoted on p. 203

plus âgées, en croissance active, le kyste, détruit antérieurement, n'abrite plus que la région postérieure; le calice chitineux devenu plus épais emboîte étroitement l'extrémité postanale du parasite, allongée en un court siphon respiratoire bisegmenté.... La structure histologique de ces organes permet en effet d'affirmer leur nature trachéenne. A la base, les cellules hypodermiques sont abondamment développées et en plusieurs couches. La sécrétion chitineuse ne forme plus, par suite, un simple filament spiral, mais une couche continue, épaisse et noire, de chitine: c'est cette région qui constitue proprement le calice. Antérieurement, l'épaisseur de la paroi kystale s'atténue, comme par étirement de la formation précédente, jusqu'à se réduire à une mince couche chitineuse incolore où l'on ne distingue plus que quelques îlots de cellules hypodermiques, les débris de mues s'ajoutent à l'ensemble."

In *Bucentes geniculata* we have found structural relations between host and parasite similar to those cited above (Pl. XIV, figs. 1, 3, 4, 5, 6, 7 and 8).

The larva of *B. geniculata* lives in the body cavity of *Tipula paludosa*. We have not, so far, found it free in this situation, but always attached to one of the main tracheal trunks of its host by means of a chitinous sheath-like structure similar to that described by the observers quoted.

At its junction with the trachea of the host, and for a short distance along its length, this sheath is thicker and of a dark brown colour. Beyond this, it is membranous in appearance and completely encloses the parasite. Usually, however, and more particularly in the older larvae, the head end of the parasite is extruded from the sheath freely into the body cavity of the host.

Recalling Pantel's description and figures of the genesis of the funnel, in which he shows that the hooks at the posterior end of the parasite are utilised to perforate the skin of the host, it seems probable to us that the attachment between parasite and the tracheal system is established in a similar manner in *Bucentes geniculata*. The body spines of the first-stage larva appear well suited for such a purpose. At first the hind end of the parasite is adherent to the host at the place where the inflammatory reaction is developed, and its skin becomes incorporated with the funnel. On moulting taking place, the larva leaves this portion of its cuticle and retracts away, leaving a clear space in this area. The relation thus set up between parasite and host involves the perforation of the host's tracheal system and establishes a common respiratory system for both. Within the sheath there are always to be found the mouth-parts and spiracles of the previous moults. In this way we have obtained from third-stage larvae two sets of moulted mouth-parts and both anterior and posterior spiracles.

DESCRIPTION OF LARVA.

First-stage Larva. As in almost all Cyclorrhaphous Diptera, the larva passes through three stages separated by two moults. The first-stage larvae obtained measured about 1.5 mm. Thirteen segments could be distinguished. The head, as is usual in Cyclorrhaphous Diptera, is divided anteriorly by

a deep median groove into two lateral lobes each of which bears a rudimentary bell-shaped antenna.

All the segments bear black chitinous hooks with the points directed backward. On the cephalic and prothoracic segments are several series of hooks, but on the remaining segments, these are smaller and fewer in number. Each of the intersegmental grooves of the abdomen is margined by hooks whose points are oppositely directed on each side of the groove.

The larva is metapneustic at this stage, the post-abdominal spiracles being terminal. These communicate with two lateral tracheal trunks.

The mouth-parts differ considerably from those of the later stages. The cephalo-pharyngeal sclerite is strongly chitinised, its posterior margin being deeply embayed. Anteriorly it is prolonged as a slender bar and terminates in a wedge-shaped vertical plate which protrudes from the mouth. Ventro-posteriorly to this terminal wedge, there lies a free sclerite which is probably homologous with the dentate sclerite of the second and third stage larvae (Pl. XIV, fig. 10).

The Second-stage larva is about 3 mm. long (fig. 3). All the segments bear small chitinous hooks, so directed that each intersegmental area is bounded by two sets of hooks, one set pointing backward, the other forward. These hooks are few in number, each segment bearing only one or two rows. Besides these all the segments bear several series of blunt transparent spines.

The prothoracic spiracles (fig. 25) terminate in five or six papillae. The post-abdominal spiracles are borne on two tubercles, each having three clefts surrounded by peritremes. On the outer border of each peritreme lies a white spot, the opening of the perispiracular gland (figs. 18 and 19).

The bucco-pharyngeal apparatus is similar to that of the third-stage larva but only half the size (fig. 11).

Third-stage Larva. The full-grown larva, when ready to pupate, is about 8.5 mm. long and 1.75 mm. in diameter. There are two cephalic segments, the first bearing rudimentary bell-shaped antennae while the second on its ventral surface bears a patch of backward pointing chitinous hooks. Behind the cephalic segments, eleven segments can be distinguished. Of these three are probably thoracic and eight abdominal. On the ventral surface these segments bear two series of similar hooks, one on the anterior margin pointing backwards, the other on the posterior margin pointing forwards. Each intersegmental groove is therefore bounded by two series of hooks pointing in opposite directions. These hooks are of two kinds—small, sharp and chitinous, and large, blunt and transparent. The small chitinous hooks are borne on the second cephalic segment and on the last three abdominal segments where they are directed forward (fig. 4).

The tracheal system consists of two longitudinal trunks, united posteriorly by a commissure, having along their length branching lateral tracheae.

The prothoracic spiracles, which emerge on the anterior margin of the first thoracic segment, are fan-shaped with from six to eight lobes. In some

cases the prothoracic spiracles show a different number of lobes in the same larva, *e.g.* one spiracle has seven, the other eight lobes (figs. 20-24).

The post-abdominal spiracles are borne on two tubercles arising from the terminal segment. These tubercles are surrounded by a chitinous hoop which is open and flattened on the inner side. Each tubercle is again bilobed, each secondary lobe opening to the exterior by two slits. Thus within the chitinous hoop there lie four slits. These slits are surrounded by oval peritremes whose internal border is dentate. The peritremes lie so that their axes converge towards the inner border of the collar. The spiracle tubercle shows two white spots, the openings of perispiracular glands (fig. 17).

The spiracles communicate with the tracheal trunks through a "felt-chamber" which is a short tube filled with a spongy chitinous structure.

On the ventral surface of the last abdominal segment lies the anus in the form of a cleft with a protuberance on each side. The posterior end of the larva moves freely in the chitinous funnel, and, as far as has been observed, the post-abdominal spiracles are never closely adpressed to the host trachea but the air from the host trachea is drawn into the chamber formed by the adhering chitinous funnel where it is at the disposal of the larva.

BUCCO-PHARYNGEAL APPARATUS.

The complete masticating apparatus of the mature larva of *Bucentes geniculata* consists of a number of paired sclerites, the members of each side articulating with one another to form a united whole (figs. 12 and 13).

Overhanging the oral aperture is a pair of strong curved hooks, the *mandibular sclerites*. Dorso-posteriorly these have a dentate process, while ventro-laterally they bear a blunt wedge-shaped process. A ventral view shows the mandibular sclerites to be united by a cross bar. Each ventro-lateral process is perforated by a minute pore.

Articulating with the posterior border of the mandibular sclerites are the *hypostomal* sclerites which are united ventrally by a transverse bar. Between the mandibular and hypostomal sclerites there lies ventrally a fused dentate sclerite which is perforate.

The posterior extremities of the hypostomal sclerites articulate one on each side with the *cephalo-pharyngeal sclerites*, which have each a slight anterior rectangular prolongation, joining it to the hypostomal sclerite of its side. The cephalo-pharyngeal sclerites are prolonged dorsally, into wing-like processes which are perforate near their outer edges, and ventrally, into a stout somewhat rectangular posterior process which has a curved incision at its extremity.

The cephalo-pharyngeal sclerites articulate ventrally with a broad chitinous plate, the floor of the pharynx, as do also the extremities of the posterior ventral processes.

The Puparium (fig. 14). The puparium is brown in colour, barrel-shaped and with clearly marked segments. Each segment bears a double series of

hooks, one series pointing backward, the other forward. The rounded contour of the first segment is broken by the slightly projecting anterior spiracles, which are directed laterally outward. Posteriorly the puparium narrows distinctly into a short tubular portion which terminates in two bifurcated lobes, the post-abdominal spiracles. In the intersegmental space which separates the first and second abdominal segments are a pair of holes situated on the latero-dorsal side.

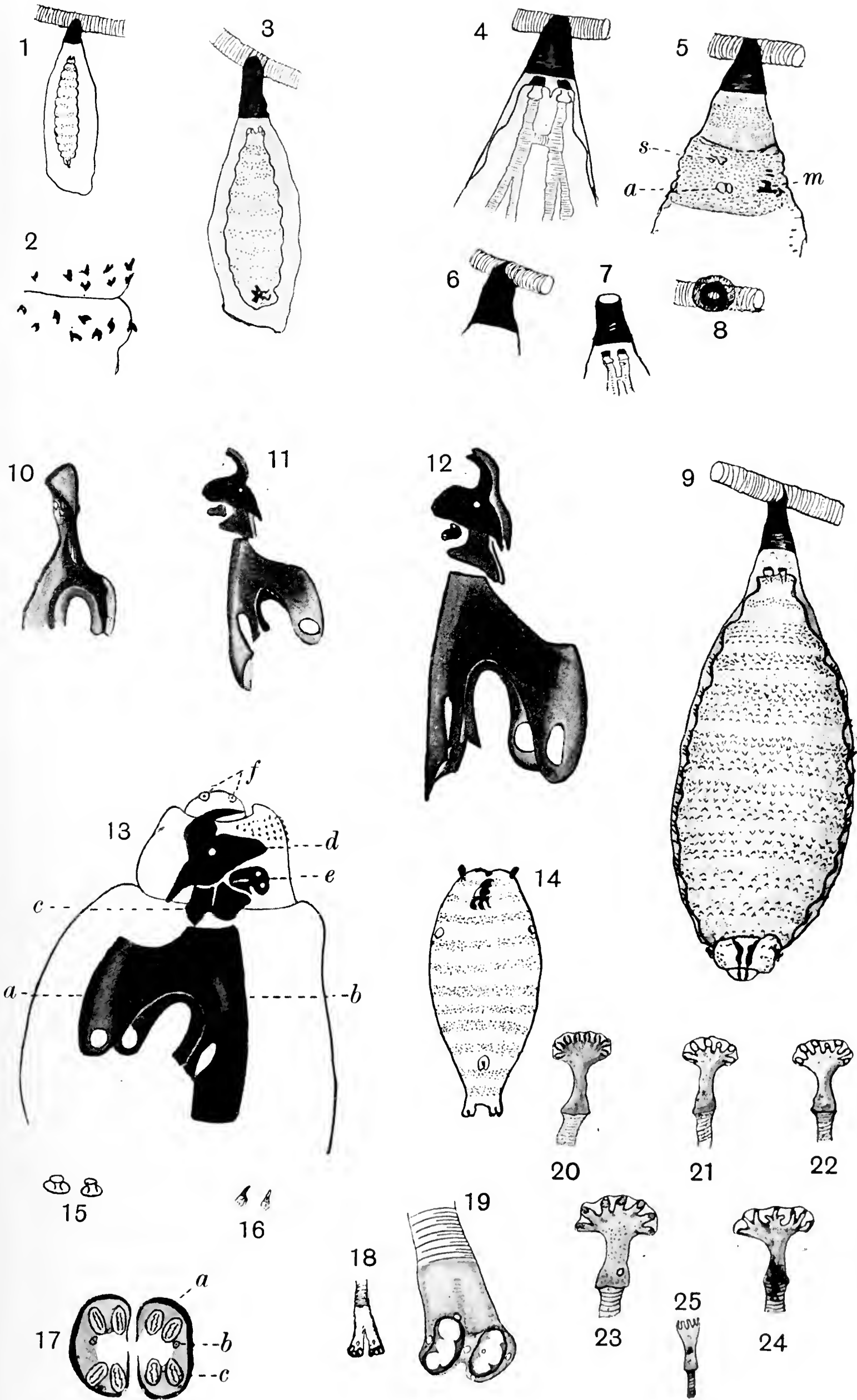
In a future paper we hope to deal with certain gaps in the foregoing account of the life history and to give a description of the structure and habits of the adult fly.

ACKNOWLEDGMENT.

We desire specially to express our thanks to Dr D. Keilin, of the Quick Laboratory, who has generously placed his extensive knowledge of the literature at our disposal, and also been most helpful with friendly criticism and suggestions. We have further found his recent work on the life-history and larval anatomy of *Melinda cognata* Meigen, parasitic in the snail *Helicella* (1919), of much assistance in determining the morphological details of the various larval stages.

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EXPLANATION OF PLATE XIV.

- Fig. 1. First-stage larva within the sheath.
Fig. 2. Intersegmental area of first-stage larva showing spines.
Fig. 3. Second-stage larva within the sheath.
Figs. 4, 5, 6, 7 and 8. Different views of attachment of larva of *B. geniculata* to its host; Fig. 5 shows the terminal part of sheath containing moult of second larval stage. The post-abdominal spiracles (*s.*) and buccal apparatus (*m.*) of the second-stage larva can readily be made out; fig. 7 shows the sheath detached from the trachea, and fig. 8 shows the attachment to trachea (end view).
Fig. 9. Third-stage larva showing anterior end protruding from sheath.
Fig. 10. Bucco-pharyngeal apparatus of *B. geniculata*, first-stage larva.
Fig. 11. Bucco-pharyngeal apparatus of second-stage larva.
Fig. 12. Bucco-pharyngeal apparatus of third-stage larva.
Fig. 13. Head and bucco-pharyngeal apparatus of third-stage larva; *a.* dorsal process; *b.* cephalo-pharyngeal sclerite; *c.* hypostomal sclerite; *d.* mandibular sclerite; *e.* dentate sclerite; *f.* rudimentary antennae.
Fig. 14. Puparium.
Fig. 15. Rudimentary antennae.
Fig. 16. Chitinous segmental hooks of third-stage larva.
Fig. 17. Post-abdominal spiracles of third-stage larva; *a.* chitinous hoop; *b.* opening of peri-spiracular gland; *c.* peritreme.
Figs. 18 and 19. Post-abdominal spiracles of second-stage larva, showing three peritremes.
Figs. 20-24. Prothoracic spiracles of third-stage larva.
Fig. 25. Prothoracic spiracle of second-stage larva.

A SURVEY OF CAWSTON'S SPECIES OF SOUTH AFRICAN CERCARIAE.

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(With 4 Text-figures.)

FOR several years Dr F. G. Cawston of Durban, Natal, has been making examinations of various mollusks taken from the rivers and pools of the Transvaal and Natal in order to discover the fluke infection of the region. These examinations have been extensive as regards the number of individuals of each species examined, ranging up into the thousands for the more common gasteropods. The investigations have been carried on through the various seasons of the year so that the data are representative. I have been enabled to re-examine much of this material through the courtesy of Dr Cawston, who has also placed at my disposal many of the biological data concerned with these researches.

It is common knowledge that the mollusk is the obligatory host of the larval fluke; that in regions where the geological or biological conditions are unfavourable for molluscan life trematodes do not occur; and that one usually expects to find fluke infection where molluscan life is abundant. But while the presence of the mollusk is an essential factor in the distribution of fluke diseases, it does not always follow that an abundance of trematodes follows an abundance of mollusks. Thus in certain parts of Japan and in the Yangtse Valley, China, fluke infection of man and other animals is common. Yet in North China, where the sanitary conditions are equally bad, trematodes are relatively rare.

Taken as a whole, one is impressed with the large number of individuals of such species as *Lymnaea natalensis* and *Physopsis africana* which Dr Cawston has examined. Infection is heavy in April and decreases toward July as the cold season comes on. Infection of *L. natalensis* is relatively light in the Transvaal and heavy at Durban. The number of infected individuals of *P. africana* is moderately few in both Natal and across the Vaal. Linked up with this fact is the paradoxical one that *P. africana* is infected with the highest number of species of cercariae (nine) and is the only host known for six of these species. *L. natalensis* has the next highest number of species (six)

of which three are specific. *Planorbis pfefferi* from Durban has a high infection which continues into the summer.

In all, 15 valid species may be recognised from the data in the writer's hand. Three of these have been recorded from the Transvaal alone, nine have been found solely in Natal, while three species are common to both regions. The larva of *Schistosoma haematobium* is apparently ubiquitous, having been recorded from Rustenburg, Pietermaritzburg, Magaliesburg, Mulder's Drift, Nijlstroom, Durban, and along the coast north of Durban.

Of these species the largest group is that of the furcocercous cercariae, containing the two human parasites, cercariae of *Schistosoma haematobium* and of *S. mansoni*. The second largest group, that of echinostome larvae, has no species that is now known to infect man in that region of the world. Either of the remaining species or others yet undescribed may be correlated with the adult liver flukes of sheep, and with monostome and amphistome infection in cattle in South Africa.

The life history of the schistosome is comparatively simple, as Leiper (1915), Manson-Bahr and Fairley (1920) and others have conclusively shown. The larva passes directly from the mollusk to the definitive host. Gilchrist (1918) has likewise shown that *Cercaria comma* is genetically related to *Distoma luteum* Gilchrist which is found in the frog. But analogy from life histories of some of the other groups leads one to believe that the solution of the problem in every case is not so simple, and that a second intermediate host may be expected.

A synoptic table of the valid species collected by Dr Cawston in the course of his examinations, together with the record of Gilchrist (1918), is included in this paper.

Material which the writer has recently had an opportunity to examine permits a description of *Cercaria pigmentosa* Cawston, previously inadequately described, and a new species of echinostome larva, *Cercaria 30-acanthostoma*, previously confused, perhaps, with *Cercaria catenata* Cawston, 1917.

***Cercaria pigmentosa* Cawston 1919.**

HOST: *Lymnaea natalensis*.

HABITAT: Natal.

This species of cercaria is the first larva of its kind to be described from Cawston's material (Fig. 1). The body is distinctly cordate with the apex directed forward. The head region is slightly protruded from the rest of the body. The body measures $333\mu \times 281\mu$. The tail is about two and one-half times the length of the body when fully extended and has a proximal diameter under these conditions of 56μ . Both body and tail are entirely covered with minute spines. The mouth is ventral in position. It has a diameter of 50μ . It leads almost directly into a small oesophagus surrounded by a small but powerful sphincter. Behind this organ the digestive tract forks to form two

stubby limbs which reach to the subdistal region of the body. The acetabulum lies in the middle third of the body. It measures 43μ in diameter and is very muscular.

The excretory bladder is a conspicuous non-muscular organ with cornua extending forward and a median vessel running backward into the distal region of the tail.

The conspicuous flocculent masses from which the larva derives its name are not pigment masses at all, but dense creamy semi-opaque vitelline follicles which with the most careful treatment remain unstained. Near their posterior limits they are connected with the medially lying germ glands by a pair of transverse vitelline ducts. The germ masses are found to contain evidences of ovary, receptaculum, Laurer's canal, vagina and seminal ducts.

The redia in which the cercaria develops is a large sacculate body with a pair of so-called feet near the posterior end and a distinct collar anteriorly. The oral sphincter is extremely small. The redia is covered with minute spines.

The cercaria is provided with abundant material for encystment. The transfer to the subsequent host probably occurs after the fluke emigrates from the mollusk.

Cercaria 30-acanthostoma nov. spec.

HOST: *Physopsis africana*.

HABITAT: Lyndenham, Natal.

This cercaria is the fourth echinostome of Cawston's collection to be described. It differs markedly from *Cercaria arcuata* and *C. constricta*. While it more nearly resembles *C. catenata* it differs from that species in many significant points.

Cercaria 30-acanthostoma measures $300\mu \times 130\mu$. The tail is slightly longer than the body. It measures 41μ at the base and tapers gradually toward the tip. The body but not the tail is covered with flat rhomboid scales closely appressed to the integument (Fig. 4). There is a circlet of 30 collar spines with blunt points and bases deeply inserted in the integument (Fig. 3). The oral sucker is inclined ventrad. It measures 38μ in diameter. The acetabulum, which lies behind the middle of the body, measures 45μ by 54μ . Between the oral sucker and the pharynx is a slight prepharyngeal sphincter. Behind the pharynx is a long oesophagus which normally forks just in front of the acetabulum. The coeca reach to the subdistal region of the body, as in most echinostome larvae.

The excretory bladder is large and distended. The pore opens posteriorly. A median cornu connects the bladder with the main collecting tubules. The latter are somewhat convoluted (Fig. 2). They contain no excretory granules. Running backward from the bladder is the main-collecting tubule for the tail. It bifurcates at the beginning of the distal fourth of that organ each fork of which opens externally. This caudal canal corresponds somewhat to the similar organ in *C. catenata*.

DISCUSSION.

With the larval stages of the more common species of flukes established for this portion of South Africa, the next problem in hand involves the discovery of the adult flukes in the same locality, flukes which possess gross

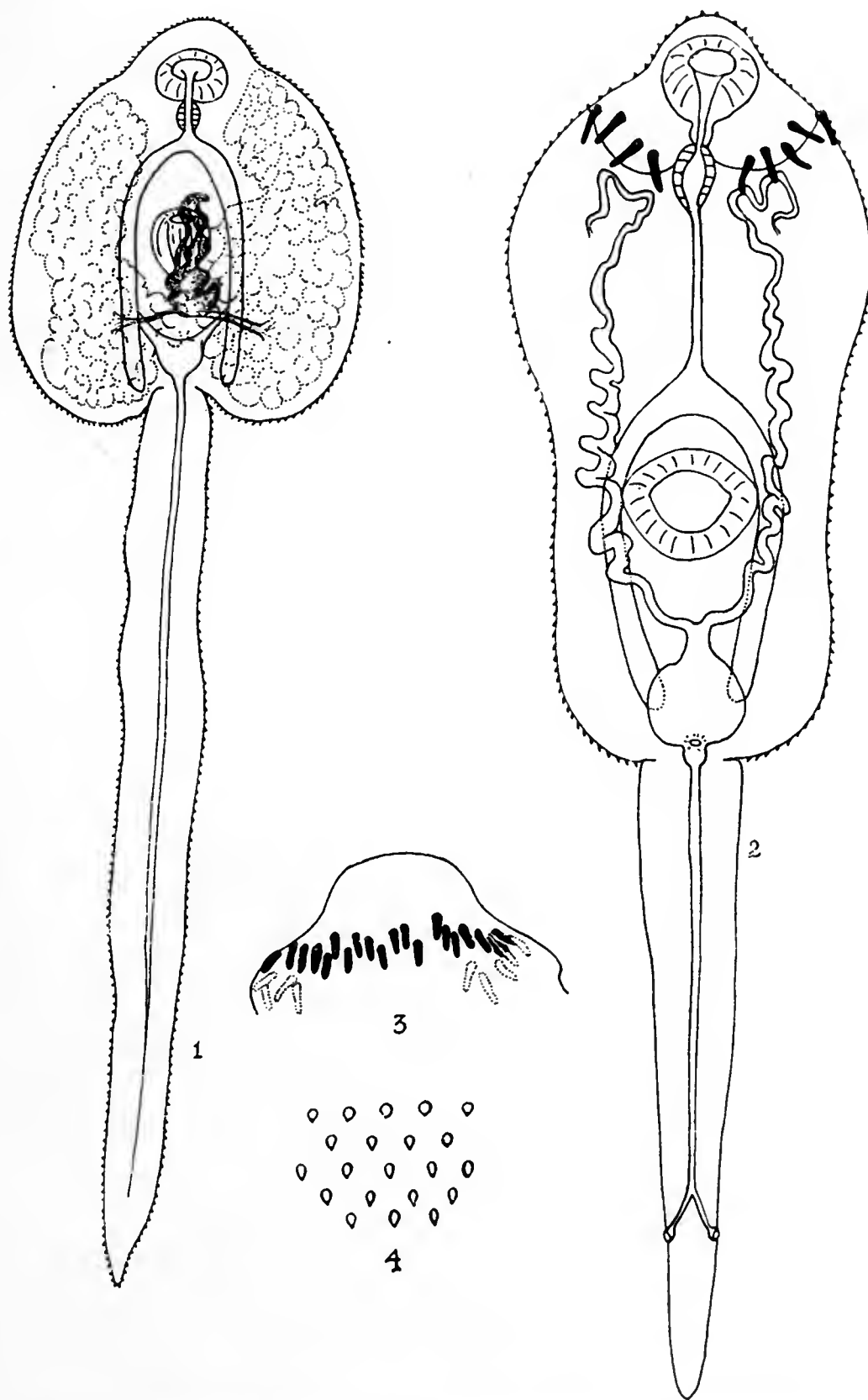


Fig. 1. Ventral view of *Cercaria pigmentosa*, showing digestive and reproductive systems. $\times 57$.

Fig. 2. Ventral view of *Cercaria 30-acanthostoma*, showing digestive and excretory systems. $\times 115$.

Fig. 3. Dorsal view of collar spines of *Cercaria 30-acanthostoma*. $\times 115$.

Fig. 4. Integumentary spines of *Cercaria 30-acanthostoma*. $\times 242$.

characters showing likely correlation with these larvae. It must be held in mind continually that many of the larval organs are *larval only* and disappear with metamorphosis; likewise, that some of the adult structures are not

recognisable in the larva. There are other characters, however, which are phylogenetically constant. These may be depended on in demonstrating the correlation.

It is not within the province of this paper to detail these fundamental homologous characters. However, the writer does desire to urge that these morphological characters be used hand in hand with the experimental work in investigating life histories, for neither one nor the other may be used alone without the danger of misinterpretation.

Table giving important biological data on South African Cercariae.

Group	Species	Described by	Date	Host	Locality
Amphistome	<i>C. frondosa</i>	Cawston	1918	<i>Isidora schakoi</i>	Potchefstroom
				<i>Isidora schakoi</i>	Stellenbosch
Monostome	<i>C. fulvoculata</i>	Cawston	1919	<i>Lymnaea natalensis</i>	Durban
Distome					
(Echinostome	<i>C. catenata</i>	Cawston	1917	<i>Lymnaea natalensis</i>	Magaliesburg
				<i>Lymnaea natalensis</i>	Durban
				<i>Planorbis pfefferi</i>	Durban
				<i>Physopsis africana</i>	Durban
	<i>C. arcuata</i>	Cawston	1918	<i>Isidora schakoi</i>	Klerksdorp
				<i>Lymnaea natalensis</i>	Durban
	<i>C. constricta</i>	Faust	1919	<i>Physopsis africana</i>	Durban
	<i>C. coma</i>	Gilchrist	1918	<i>Isidora tropica</i>	Muizenburg Lake
	<i>C. 30-acanthostoma</i>	Faust	1920	<i>Physopsis africana</i>	Lyndenham
Xiphidio-cercariae	<i>C. cawstoni</i>	Faust	1919	<i>Physopsis africana</i>	Maritzburg
				<i>Lymnaea natalensis</i>	Maritzburg
				<i>Planorbis pfefferi</i>	Durban
Leptocercariae	<i>C. pigmentosa</i>	Cawston	1919	<i>Lymnaea natalensis</i>	Natal
Furcocercariae	<i>C. of Schistosoma</i>	Cawston	1915	<i>Physopsis africana</i>	Rustenburg
	<i>haematobium</i>				Maritzburg
					Magaliesburg
					Mulder's Drift
					Nijlstroom
					Durban
					Ottawa
					Lyndenham
	<i>C. of Schistosoma</i>	Faust	1920	<i>Physopsis africana</i>	Durban
	<i>mansoni</i>				Ottawa
					Lyndenham
	<i>C. gladii</i>	Cawston	1918	<i>Isidora schakoi</i>	Potchefstroom
	<i>C. secobii</i> sp. inq.	Cawston	1915	<i>Physopsis africana</i>	Maritzburg
	<i>C. oculata</i>	Cawston	1917	<i>Physopsis africana</i>	Durban
	<i>C. parvoculata</i>	Cawston	1919	<i>Physopsis africana</i>	Durban
	<i>C. bilharziellalunata</i>	synonym of <i>C. oculata</i>			
	<i>C. spinosa</i>	synonyms of cercariae of human schistosome species			
	<i>C. crispa</i>				

OBSERVATIONS ON THE GEOGRAPHICAL AND ETHNOLOGICAL DISTRIBUTION OF HOOKWORMS¹.

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DURING the course of investigations, primarily directed to a study of the effects of hookworm infection on people in different parts of the tropics, I have been struck with the peculiar geographical and racial distribution of the two common species of hookworms infecting man, and with its bearing on certain ethnological problems.

The observations I have made, in so far as they are related to anthropological questions, are as follows: The races of mankind, living in tropical and subtropical regions, are infested with one or more species of hookworms. In the migrations of these peoples the immigrants have carried their peculiar species of hookworms into regions occupied by people having a different worm-species-content, and by an examination of the intestinal worms of a people, the geographical and ethnic origin of their hosts can, within certain limits, be divined. I refer particularly to migrations within 35° N. and 30° S. latitudes, for when migrations are made into colder climates the hookworm infection is ultimately lost through inability of the embryos to persist during the phase of their life cycle spent in the soil.

There exists the deepest ignorance in regard to the migrations of people that have taken place in prehistoric times and also in regard to those within historic times where no records have been left.

But at present, in tropical countries, certain movements of populations are going on in response to a demand for agricultural labourers.

Large numbers of Tamils and Malabarais have been recruited to Malaya to work on rubber estates. Thousands of Chinese go annually to the same country to work in the tin mines. A great many North Indians from Calcutta are being employed under indenture in Fiji and British Guiana on cane and rice plantations, there is a Javanese colony in Dutch Guiana. Japanese are colonizing in the State of São Paulo, Brazil.

The origin and movements of these people are well known. They have brought enormous numbers of hookworms into the countries where they are employed. A study of the worms expelled from these people as well as those

¹ This work for the most part was done under the auspices of the British Colonial Office and with the support of the Rockefeller Foundation.

expelled from the autochthones in the countries in question discloses some interesting facts.

Taking the island of Viti Levu, the autochthones show almost a pure infection with one species of worm, *Necator americanus*. No specimens of *Agchylostoma duodenale* were encountered in Fijians except among those that had lived in towns, or near plantations occupied by East Indians.

Among indentured East Indian coolies from Calcutta 21.4 % of the worms expelled were *A. duodenale*, the rest were *N. americanus*.

Wherever Fijians were living near East Indians, they were found to have become infected with *A. duodenale*, derived from the latter race.

Infection by *A. duodenale* is becoming widespread among town-dwelling Fijians.

After East Indians have lived in Fiji for 10 to 15 years or more they lose a good many of their *A. duodenale* and disclose a relatively larger proportion of *N. americanus*.

In Malaya the Malays were found to harbour nearly a pure culture of *N. americanus*, for 98.99 % of their worms were of this species, the remainder being *A. duodenale* and *A. ceylanicum*. This may be considered the formula of the country. Chinese coolies coming in to Malaya bring a worm formula of 35 to 85 % *A. duodenale*, the remainder being *N. americanus*. However, among the generation of Chinese born in Malaya—Straits born Chinese, who have often taken up Malay customs—eating with their fingers, etc., and who may have dropped Chinese customs and associates to a greater or less extent, it was remarkable to find that they had dropped also the Chinese worm formula as well, and taken up one like that of the people of the country. At the same time the influx of Chinese coolies with their large ancylostome index is gradually adding an increasing number of hookworms of this species to the common species of the natives of the peninsula.

The effect of migration into a country whose people have a different index from that of the immigrants, is to make the immigrant take on the worm index of the autochthones, while the index of the latter is more or less modified by the implantation of the worm species of the immigrant.

This was very clearly shown in Fiji.

The autochthonous population as represented by the people of Nasoqo, a remote and inaccessible interior mountain village, were found to be infected with *N. americanus* (six specimens of *A. ceylanicum* were encountered). Not a single specimen of *A. duodenale* was taken. The ancylostome index of the autochthones then must be considered as nil.

In the villages near Nausori where the autochthones are exposed to infection from soil polluted by East Indians, they were found to be harbouring some *A. duodenale*. Thus ten Fijians treated, were found to have 1309 hookworms of which 1246 were *N. americanus* and 63 *A. duodenale* (there were six dog-worms *A. ceylanicum*).

The ancylostome index of these town-dwelling Fijians was 4.8 % and

represents infection derived from North Indian sources, for the ancylostome index of the Calcutta men was 27, while that of uncontaminated Fijians was nil.

Here we are to observe two tendencies. First: a tendency for the stranger to part with some of his worms and to acquire a worm formula like that of the natives. Second a tendency for the natives to acquire certain worms from the strangers and thus modify the primitive formula of the region or race. This is actually taking place in Malaya, Fiji, Guiana and Brazil. Changes analogous to these that are taking place under our very eyes, may be assumed to have taken place centuries ago among other peoples, and if among a people to-day whose worm formula is 99 % *Necator* we encounter a group who are harbouring 15 % *A. duodenale* or any notable number of that species we are entitled to assume that the hookworms of the latter people are derived from an alien stock.

The hookworms encountered in man are:

<i>Agchylostoma duodenale.</i>	<i>Necator americanus.</i>
„ <i>ceylanicum.</i>	
„ <i>braziliense.</i>	

These worms probably all have identical life cycles. *A. duodenale* and *N. americanus*¹ are obligate parasites for man, as they are not found in any of the animals.

A. ceylanicum and the less known *A. braziliense* are commonly found in dogs in certain tropical lands particularly in the Old World.

The adult sexually differentiated worms live in the small intestine and the females produce many ova which pass out in the faeces.

Embryos from the ova-infected faeces develop in the soil under suitable atmospheric conditions of warmth, moisture and oxygen.

The embryos penetrate the skin of the feet of people visiting polluted places and after traversing the skin, venous blood channels, right heart, lung and trachea, they reach the intestinal tract where they take up lodgement in the lumen of the small intestines, holding on to the mucous membrane by their strong armed mouths, and remaining in the host as long as seven or eight years.

While in general it has been recognized that two species chiefly were to be found in man, no survey has been made of the species actually harboured by the different races of mankind or by the inhabitants of different zones, regions or localities.

The reasons are obvious. Anti-hookworm campaigns are of very recent date and the doctor in charge is usually concerned merely in ridding the patient of worms and ameliorating his physical condition.

The washing of faeces after employing a vermicide, and the culling and counting of thousands of worms under field conditions is not an easy or enticing vocation.

¹ *Necator americanus* has been found in a gorilla by Leiper and Looss.

Rarely however it has happened that the careful counting of hookworms expelled from a large number of people was necessary to the elucidation of some intensely interesting and very important medical and public health problems. Such problems were presented to me and my colleagues, Doctors M. B. Barber, H. P. Hacker, M. B. Barnes and W. G. Smillie. In the prosecution of our work the drudgery of the task was alleviated by the extraordinary interest aroused as the solution of the problems appeared to present themselves.

DISTRIBUTION OF *AGCHYLOSTOMA DUODENALE*.

A. duodenale is distributed to all those countries lying in Eurasia, south of 35° N. latitude and north of 20° N. latitude. In Europe, as in Cornish mines and in Mediterranean countries and Egypt, it may be the sole species found; nearer the Tropic of Cancer it is associated with increasing numbers of *N. americanus*. In warm mines the worm may be found farther north than 35° N.

This species has been introduced into the American continent and the Antilles in historic times by coolies from India and Java, by Mediterranean and Levantine people into Brazil and other parts of North, Central and South America, and by Japanese and Chinese.

It has been introduced into Fiji by Indian coolies, and into Polynesia, the Philippines, and Australia, by Chinese, Japanese and Portuguese. In Europe it has been encountered in Italy, Sicily, Sardinia, Spain, Austria, Hungary, Serbia and Bulgaria. Farther north it is common among miners as in Cornwall, Belgium, Liège, Mons and Charleroi. In France it occurs in the Loire Basin, also in the mines of Germany, Poland and Silesia. Severe infections are encountered in Egypt.

While in general the species *A. duodenale* is distributed in the cooler latitudes north of 20° N. latitude, there seems to be no reason other than the lack of opportunities for implantation, for its relative absence in equatorial regions.

Its absence is only relative for I encountered large numbers of this species in Java and Malaya among people who acquired the infection within 3° N. and 8° S. latitudes. It is evident therefore that there is nothing in an equatorial climate inimical to the development of the species in the soil and that the incidence of each species is purely a matter of implantation on soil equally favourable to each.

DISTRIBUTION OF *NECATOR AMERICANUS*.

This species is found in largest proportion in Eurasia, Africa, Indonesia and Polynesia, south of 20° N. latitude.

In regions near the tropic of Cancer it is associated with *A. duodenale* but farther south, as in parts of Indonesia, Fiji and South Africa, it is encountered alone.

This species has been introduced into America within historic times by Kaffir slaves from Africa and to a certain extent by the East Indian coolies from British India and Java. It is the species most commonly found in America and has been spread from Virginia to Argentina.

It is possible that either or both species have also been introduced into the American Continent from Asia, Indonesia or Polynesia by voyagers or storm-tossed fishermen. This is an enticing subject for future research.

The American Continent may have been peopled (a) from Asia by way of Behring Straits, (b) from Asia or Indonesia across the Pacific, or (c)¹ from Polynesia across the Pacific.

In the case of (a) cold would prevent the continuance of infection and the migrants would arrive free from hookworm, unless of course the average temperature of the Straits during migration was equal to that of North Carolina at the present time, for the latter temperature is the northern limit of autochthonous hookworm infection in America. In the case of (b) we might expect to find either or both *A. duodenale* and *N. americanus* among the Amerinds or in such representatives of them as may have descended from (b). In the case of (c) we would expect to find only *N. americanus*.

A survey of the species of hookworms harboured by Amerinds unexposed to infection from other sources, is urgently required to clear up some of these points.

In Panama the population composed of Meztizos and Zambos was found to be almost exclusively infested with *N. americanus* which may have been derived in the first instance from Negroes.

The reports of the Field Director of the International Health Board working in Central America show that the Indian (Amerind) population is infested with hookworms but the observations have been carried out in localities and among Indians who have been living in an environment contaminated with excreta of Negroes and East Indians which contain embryos of both *Necator* and *Agchylostoma*.

It will be necessary to search for tribes in inaccessible and remote places when it is desired to ascertain the primitive worm species index of uncontaminated Indians (Amerinds).

GEOGRAPHICAL AND RACIAL DISTRIBUTION OF HOOKWORMS.

AMERICA.

The species of hookworms encountered in the Southern States according to Dr Stiles is *N. americanus*, this was derived no doubt from the introduction of negro slaves from Africa.

¹ Cowan (1910), *The Maoris of New Zealand*, Christchurch, N.Z., records that in 1909 a Chinese fishing junk picked up off the island of Chu San a party of three South Sea Islanders who were adrift in a canoe, they had mother-of-pearl shell fish-hooks and other South Sea fishing tackle with them, and had been blown and drifted fully 2000 miles from a German possession in the Western Pacific.

No survey of the hookworms of North American Indians has yet been made. It is extremely important that this be undertaken without delay.

PANAMA.

In Panama there were two types of infection: (a) pure infections of *N. americanus* encountered among the Panamans and probably derived from African negro sources, (b) mixed infections in which both *A. duodenale* and *N. americanus* were found. This type of infection occurred chiefly among West Indians who had been exposed to infection in their native islands from East Indian coolie sources. But it also occurred to a slight extent among natives of Panama who had been exposed to infection from West Indian negroes resident in the Canal Zone since the commencement of canal operations.

BRAZIL. *State of São Paulo.*

The species most commonly found is *N. americanus* but the proportion of *Agchylostoma* to *Necator* varies in different communities depending on circumstances.

At *Itatiaia*, in a former coffee plantation where many African negro slaves were worked years ago, the ancylostome index is 1.5.

At *Rezende*, which was formerly a detention and distribution centre for Italian, Portuguese and Spanish agricultural colonists, the ancylostome index as we might expect is much higher, 11.2, and is evidently due to the seeding of the soil with *A. duodenale* by Mediterranean people.

At *Brodowski* the high ancylostome index of the Japanese is being markedly altered by residence among the native Brazilian agriculturists who are so heavily infested with *N. americanus*.

Nothing has as yet been done to determine the species formula of the aborigines of South America.

Great care must be exercised in selecting tribes who live in inaccessible regions unvisited by foreigners. It should not be hard to do this in the interior of the continent or in Andean villages.

EUROPE.

The species encountered in Europe is *A. duodenale* except in those immigrants returned from Brazil or the United States.

The hookworm found infecting Cornish miners was *A. duodenale* (Boycott).

Isola (1904) examined 11,000 specimens collected by Parona from upper Italy and all were *A. duodenale*.

AFRICA.

Northern Africa.

The species found in Egypt and Northern Africa appears to be exclusively *A. duodenale* for this was the sole species encountered in the large mass of material passed under review by Looss in Egypt.

Equatorial and South Africa.

In a number of autopsies I performed in Johannesburg on Kaffirs from Mozambique, the only species encountered was *N. americanus*.

Leiper, Looss and Fülleborn found this species in South Africa and among Pygmies in the Cameroons, respectively.

The Kaffirs¹ appear to be parasitized exclusively by this species. The introduction of slaves from Mozambique infested with *Necator* will account for the exclusive presence of this species in the Southern States of America.

ASIA—INDONESIA.

Malays. The natives of the Malay Peninsula.

The Malays live in kampongs or native villages usually occupied solely by individuals of their own race. This was particularly true of the two kampongs near Kuala Lumpur where we obtained some data.

Thirty-eight boys from Kampong Malacca yielded 2262 hookworms of which 2257 were *N. americanus*, and 5 were ancylostomes, 3 *A. duodenale* and 2 *A. ceylanicum*.

In Ulu Gombak 39 boys yielded 1559 hookworms, 1546 of these were *N. americanus* and 13 ancylostomes, of these 7 were *A. duodenale* and 6 *A. ceylanicum*.

Thus the percentage of ancylostomes present in the Malay kampong boys was 0.22 and 0.8 respectively.

Taking the two groups of 77 boys as a whole as representative of what the Malay worm formula should be and considering *Necator* and *A. duodenale* only, there were 3813 worms of which 10 only were *A. duodenale*, or an *A. duodenale* index or percentage of 0.26.

Adult Malays. Adults from their occupations are often brought into contact with Chinese of which there are large numbers in the peninsula and they have more opportunities for becoming infested with the hookworms of the Chinese, who, as it will be shown, harbour very large numbers of *A. duodenale*. We may expect the adult Malays to-day therefore to show some evidences of contamination from Chinese sources.

Sixteen adult Malays were found to harbour 1138 hookworms of which 10 were *A. duodenale* and the remainder *N. americanus*, the *A. duodenale* index being 0.9. Thus we see that the autochthonous Malay population of the Malay Peninsula harbours nearly a pure culture of *N. americanus*.

Malays of the Island of Java.

Among the natives of Java there was encountered a very distinct difference between the ancylostome index of the people of West Java and that of people

¹ *A. duodenale* unquestionably is a more malignant hookworm than *Necator*. It is possible that some of the alleged immunity of the negro to the effects of hookworm infection is due to the fact that Kaffirs and their descendants are very largely parasitized by *Necator* and not by *A. duodenale*.

living east of the Tji Manoek river, this difference corresponds with a difference in the ethnic stocks in the two regions.

Worm counts were carried out among Malays in four kampongs in the city of Batavia (West Java): a total of 2935 hookworms were obtained from 92 people of which only 26 were *A. duodenale*, the remainder being *N. americanus*; the percentage of ancylostomes therefore was 0.88 a figure corresponding closely with that of the Malays of the Malay Peninsula.

In the mountainous region of the Preanger (West Java) there was an absence of *A. duodenale* which was very striking indeed.

Twenty-five Malays (Sudanese) in the dessa or village of Endil Tjhoeavglagte were found to be harbouring 1275 hookworms, and of these two were *A. ceylanicum* (derived from dogs), the rest being *N. americanus*.

In the dessa of Tjimatjam, at an elevation of 3600 feet, near the volcano Gedeh, where there was a minimum pollution of soil and of the water courses, 25 persons yielded only 150 hookworms all *N. americanus*.

In these two dessas in the Preanger the *A. duodenale* index was nil.

This absence of *A. duodenale* among 50 representative Malays in the Preanger, indicates in the most striking way that the hookworm common and proper to the people of West Java is *N. americanus* and that *A. duodenale* when found represents an extraneous infestation from some alien source.

Wishing to include in the survey some of the people of mid-Java I visited two villages near Cheribon on the North Shore:

At Gebongelir, 50 Javanese were treated, and a total of 2339 hookworms were obtained from them; of these 308 were *A. duodenale* the remainder being *N. americanus*, the ancylostome index being 13.1.

At Kalimaro, a village near by, 5140 hookworms were obtained from 24 persons; 332 were *A. duodenale* and the remainder were *N. americanus*, an ancylostome index of 6.2.

In the dessa of Kibasekan near Keboemen, not far from Djokjakarta (mid-Java), 25 persons yielded 4082 hookworms, the percentage of *A. duodenale* being 5.4.

From Krakal and Karangsari, villages in the same district, 28 Javanese yielded 10,861 hookworms of which 770 were *A. duodenale*; the ancylostome index being 7.0 %.

The distribution of *A. duodenale* and *N. americanus* in Java and the neighbouring islands is well displayed in an analysis of the results of the worm findings among the prisoners in the jail in Batavia.

In this jail there are no opportunities for acquiring new hookworm infestation. Food is dispensed in a pasteurized or sterilized state while still warm. The sanitary arrangements while primitive I think effectually prevent hookworm infestation.

Confirmatory of this is the fact that of 118 men treated who had been in the jail for periods up to seven years or more, the average number per man was found to diminish with each succeeding year of imprisonment. This is inter-

preted as meaning that the older worms die and are expelled, while there is no re-infestation or new infestation to make up this loss.

The people living in the kampongs round about outside the prison have, as we have seen, an ancylostome index of 0.88 %.

Within the jail compound the index is 9.2 % from a total count of 8638 worms derived from 118 prisoners. This indicates that the prisoners bring their hookworms in with them and do not derive them directly or indirectly from the kampong people outside the jail through polluted water or food. We are confirmed in this view on an analysis of the findings, after separating the men into groups based on the part of the Netherlands Indies they came from.

Thus there were two prisoners giving their place of origin as Batavia or the Preanger, that is, West Java. The total worm count was 130 and the ancylostome index was 0.76 which is a West Java index.

There were nine prisoners from mid or East Java. The total worm count was 755 with an ancylostome index of 7.5 %—a mid-Java index.

Taking up the prisoners from Sumatra and other islands of the Netherlands Indies the index of each place may be ascertained from the prisoners and their respective differences noted.

Madura. This small island almost touches the eastern end of Java. There were 16 Madurese prisoners from whom 1263 hookworms were obtained; the ancylostome index was 13.7 %.

Bali. This interesting island, where the Hindu religious influence is still paramount, yielded five prisoners; the worm count was 348 and the ancylostome index 4.0 %.

Lombok, a neighbouring island, with 4 prisoners and a total worm count of 479, gave an ancylostome index of 3.3 %.

Timor. This island, with four prisoners and a worm count of 100, gave an ancylostome index of 62.0 %.

Sumatra supplied 12 prisoners. The total worm count was 919 of which only 7 worms were *A. duodenale*, the ancylostome index being 0.9 %. Thus the index of Sumatra resembles that of the people of the adjoining portion of West Java and the Malay Peninsula in contra-distinction to that of the people of mid and East Java and the chain of islands extending towards Timor.

(There were two prisoners who had worm formulas resembling that of the Chinese. One of these, a Madras Tamil, had lived five years close to Chinese lines. The ancylostome index of these two men (38 worms) was 65 %. It is evident that they were infested from Chinese sources.)

Celebes. This island lies to the north of Timor and to the west of Borneo.

It supplied four prisoners yielding 523 hookworms, all *N. americanus*, the ancylostome index therefore being nil and representing what we may call the primitive Malay species formula.

This completes the data, highly interesting and important as they are in respect to the indices of the autochthonous population of Indonesia.

Unfortunately there were no Malays from Borneo, Flores or Sumbava in our treatment groups, but the evidence as it stands, points distinctly to the supposition that the Malay people living in Sumatra (their ancient home?), Malay Peninsula, Java, Celebes and all the other islands of Indonesia were originally infested with *N. americanus* whilst *A. duodenale* became superimposed upon this infection in certain localities and islands through the migration thither of an alien ethnic stock or stocks infested with a much higher percentage of *A. duodenale* than that harboured by the autochthones.

From evidence to be presented there can be no doubt but that *A. duodenale* was introduced into Indonesia from the continent of Asia and in sufficiently large numbers to have become well implanted in the people and soil of mid and East Java and of Madura, Lombok, Timor and Bali as well.

What was the ethnic source of the migrants?

It was almost certainly from some region north of about 20° N. latitude and may have been North Indian, that is from above the delta of the Ganges or thereabouts, Upper Burmah or Assam or it may have been from China.

Regular commercial relations had been maintained between India and Java since about 700 B.C., according to Oldham¹. Arab and Chinese merchants have been coming to Indonesia for centuries and from these three sources the soil of Indonesia undoubtedly has been seeded with *A. duodenale*.

But to account for the high ancylostome index encountered in mid and East Java, Madura, Lombok and Timor we must assume the migration of larger numbers than would be represented by a few traders and sailors.

A volume of people corresponding to the respective numbers of the two species of hookworms in the ancylostome index has been necessary to produce the index as we find it.

That is to say, a thousand migrants with an ancylostome index of 75 % when mixed with a Malay population eight times as large with an ancylostome index of nil will in time yield a mixed population with an ancylostome index of 8.3 %.

Buddhism is said to have reached the Indian Archipelago about 223 B.C. and there are Javan traditions that about 300 B.C. thousands of families from N.W. India and from the Kling coast were established in Java.

Java and possibly other islands in Indonesia were subjected to Hindu domination for 14 centuries or up to the 15th century A.D. The temples date from about 600 A.D. This was followed by a Mohammedan invasion. Portions of Indonesia undoubtedly became seeded with *A. duodenale* at this time.

It seems probable that the present ancylostome formula of the mid-Javanese resulted from this Hindu invasion, for the high ancylostome index is found in those districts in Java where the Hindu culture, as evidenced by the presence of temples or their ruins, was most intense, that is to say in mid and East Java.

¹ Oldham (1905), *The Serpent and the Sun*, London.

There are no chandis in West Java and but few ancylostomes there. In the Preanger they (*A. duodenale*) are absent.

Beneath the cult of Mohammedanism in Indonesia there exists the remnants of Buddhism and Brahmanism. I have seen Javanese women who were nominally moslems actually worshipping in the ruins of a Brahman temple—a Hindu God. Underneath Brahmanism there yet lurks a pretty lively animistic cult, for the “hantu” or ghost possesses considerable influence in Malay households. Malay fishermen, at any rate in mid-Java, still attempt to propitiate by offerings of food the spirit which they believe presides over the destinies of fish and of fishermen, and thus modify the proverbial fishermen’s luck.

Mohammedanism, Buddhism and Hinduism as well as Christianity have come to Indonesia from without.

It is possible that these cults in turn have been superimposed upon a pre-existing animistic cult or cults also due to outside agencies.

Perry¹ has called attention to the occurrence of stone monuments, dolmens, dissoliths, alignments and menhirs in Indonesia, notably in the Timor region, which bear certain resemblances to megalithic structures found in Burmah and Assam. He infers that the use of stone was not indigenous but was introduced from without to various parts of Indonesia. The new comers also introduced metal working, terraced irrigation and rice culture. These stone working migrants may have come from Burmah or Assam².

Whoever they were or at whatever period they made their appearance, if they contributed to the implantation of the ancylostome formula of the Timorese etc., it will be evident that they came from north of about 20° N. latitude.

This belief in the source of *A. duodenale* is based on information derived from worm findings among North Indians, Chinese and Japanese.

With regard to North Indians the information comes from two sources, North Indians examined in (a) the Malay States and (b) Fiji.

North Indians in the Federated Malay States.

These are represented by Sikh and Mohammedan Police from the Punjab. Some of these men had lived in the Federated Malay States ten or more years and their native or natural formula had possibly undergone some changes due to infection derived from Tamil, Malay and Chinese sources; however if we take only those men who had lived in the Federated Malay States ten years and under, there are 7 cases with a total of 41 hookworms, 21 of which were *A. duodenale* or an ancylostome index of 51.2 %. A second group from among hospital patients was made up of Sikhs and Bengalis, 8 persons, with a total of 222 hookworms of which 71 were *A. duodenale* or an ancylostome index of 32 %.

¹ Perry (1918), *The Megalithic Culture of Indonesia*, Manchester.

² There is however a trilithon at Haamonga, Tonga, which, according to tradition, was built by Polynesians (Samoans).

North Indians in Fiji.

Additional data and probably of greater value were obtained in Fiji from indentured and free coolies that had come directly from Calcutta. That is to say, they had embarked at Calcutta having been recruited from various places, for the most part north of 20° N. latitude—Central United and North West Provinces and speaking Hindustani—not Tamil or Telegu.

The information is of great value for there are no Chinese or other ancylostome bearing people on the island to complicate the species formula.

Thirty-four North Indians that had been brought out under indenture and had worked in the plantations five years and under were found to harbour 2480 hookworms, of which 684 were *A. duodenale* and the remainder *N. americanus*, the gross ancylostome index being 27.5 %.

The individual percentages vary from nil to 93 as is seen in the following table.

North Indians, Fiji. Years resident in Fiji.

1 year		2 years		3 years		4 years		5 years	
<i>A. duod.</i>	<i>Necat.</i>	<i>A. duod.</i>	<i>Necat.</i>	<i>A. duod.</i>	<i>Necat.</i>	<i>A. duod.</i>	<i>Necat.</i>	<i>A. duod.</i>	<i>Necat.</i>
33	11	51	5	31	17	9	17	8	27
21	197	3	38	28	98	19	83	47	74
30	20	56	36			4	29	53	347
1	6	24	247			29	64		
		31	24			8	16		
		5	6			0	17		
		17	25			1	1		
		5	2			39	3		
		15	3			3	20		
		23	8						
		43	101						
		16	7						
		20	83						
		5	90						
		1	58						
		5	16						

The free Indians or those who elected to remain in Fiji after their indenture had expired, were found after 7 to 14 years of residence to have lost a good many of their *A. duodenale* but to have become severely infected with *Necator*—the hookworm of the Fijians and of the country.

Among 35 *Free* North Indians the ancylostome index was found to be only 4.2 %—a striking illustration of the tendency of the index of the immigrants to approach that of the autochthones.

OTHER ASIATICS FROM NORTH OF 20° N. LATITUDE.

Chinese.

The Chinese fall into two groups (*a*) those born in China who came to the Federated Malay States or Java as adults and (*b*) those born in Indonesia.

Chinese immigrants to Indonesia come from the Southern Provinces and

are known as Khehs, Cantonese, Hylams (Hainan) and Hockians. The new comers or "Sinkehs" of course bring with them typical worm formulas of their native country.

"Lowkehs" or men who have returned to Indonesia after a previous visit possess formulas altered possibly by previous residence.

It is not always possible to learn with accuracy a man's previous residence, for the Oriental is very guarded in replying to questions and may have reasons for wishing to appear as a new comer, this being particularly the case in Chinese who had been banished for crime.

At St John's Island, Singapore, we treated 46 "Sinkehs" and obtained 1241 hookworms of which 420 were *A. duodenale* and 821 were *N. americanus*, thus the ancylostome index was 33.8. In this group there were ten cases of pure *Necator* infection yielding altogether 148 worms. 36.9 % of the men had indices falling within the group index of 33.8.

Among the patients treated at the District Hospital, Kuala Lumpur, there were 79 Chinese who yielded 5191 hookworms, of these 1994 were *A. duodenale* and 3197 *N. americanus*, the ancylostome index being 38.4.

In this group there were 12 cases of pure *Necator* infection and 37 other cases whose indices were below the average for the whole group. The men had lived in the Federated Malay States for periods up to 20 years and possibly to some slight extent represent infection derived from other races. On the other hand the index is very close to that of Sinkehs and the index 38.4 or 33.8 in all likelihood represents a border line or frontier index, where the species overlap.

In five fatal cases of hookworm infection the ancylostome index was 86.1 %, there having been found 3779 *A. duodenale* and 698 *N. americanus*.

Chinese born in Indonesia.

Some data were obtained on the index of Chinese born in Indonesia, few cases it is true but very interesting as showing how children of the foreigners take up the worm index of the natives of the country.

Two Straits born Chinese were examined in Kuala Lumpur. One was of the first generation, the other of the second generation born in the Malay Peninsula. 72 worms were obtained from these young men, all *Necator*, the ancylostome index being nil.

Among the Chinese treated in the Batavia jail there were two born in Java. The man who was born in West Java had 123 hookworms, one of which was *A. duodenale*, a percentage of 0.8, which is a West Java index. The other who was born in mid-Java had 57 hookworms of which 11 were *A. duodenale*, giving a percentage of 19.3 which is a mid-Java index. Rather a striking illustration of the effect of regional soil in determining the species formula.

The physical resemblances between Malays and Chinese are very strong indeed. Wallace observed this in adults and even after having become

thoroughly familiar with the two races, I was at times unable to tell which of two sunburnt boys was the Malay and which the Chinese.

Yet close as the physical resemblances such as physiognomy, physique and cranial indices are, the helminthological testimony indicates that the Malays as a whole are no recent migrant stock from Southern China.

The Malays are not Chinese who have moved southward towards the equator and mixed with a dark skinned race, for the worm formula of the Malays is distinct from that of the Chinese. If the Chinese had migrated into Indonesia in hordes the ancylostome index of the Malays would approach that of the Chinese but this it does not do.

Some Malays may have moved northward and added their complement of *Necator* to an ancylostome-bearing Chinese stock, for the Chinese of South China to-day carry large numbers of *Necator* with their ancylostomes.

The presence of *A. duodenale* in mid and East Java, Madura, Lombok and Timor is not due to the presence of the relatively few Chinese coolies, traders or shopkeepers there, for the native population greatly outnumbers the Chinese. As a result we see the Chinese that are born in Java taking on the native index.

On the other hand where large numbers of *A. duodenale* carriers are present, as among the North Indians in Fiji, they are perceptibly infecting the native Fijians, just as we may suppose the Hindus or Burmese may have infected the Javanese of mid-Java centuries ago.

A small group of Japanese women, four in number, who had lived in the Federated Malay States three years and under were found to harbour 61 hookworms of which 19 were *A. duodenale*, giving an ancylostome index of 31.1. The index of the Japanese therefore resembles that of the Chinese and North Indians and is in marked contrast with the primitive index of people south of 20° N. latitude, that is, the Tamils, Malabarais and Malays.

Thus we see that there is a solid zone lying to the north within which the people have high ancylostome indices. It is from people in this more northerly zone that *A. duodenale* was introduced into mid and East Java and neighbouring islands.

South Indians.

Tamils, Malabarais and other Davidian natives of Southern India entering the Port Swettenham Quarantine Station were found to possess a uniformly low ancylostome index.

A series of treatments were carried out by Hacker and Barber on South Indian coolies, Tamils and Malabarais at Port Swettenham Quarantine Station.

From three squads, 31 persons, 4363 hookworms were obtained, 89 of which were *A. duodenale* giving an ancylostome index of 2.0.

This low ancylostome index among South Indians was encountered with great uniformity and serves to differentiate the North from the South Indians.

Twenty-five Malabarais that had lived in the Federated Malay States but

a few months yielded 3491 hookworms of which 30 only were *A. duodenale* and the remainder *N. americanus*, an ancylostome index of 0.86.

A group of 35 Tamil coolies engaged in road repairing in Kuala Lumpur were found to be harbouring 2870 hookworms of which 59 were *A. duodenale*, the remainder *N. americanus*, the percentage of ancylostomes being 2.0.

Polynesia.

Fijians represent a mixture of two stocks, Melanesian and Polynesian.

The typical Melanesian is exemplified by the Papuan, while the Polynesian is represented by the Tongan or Samoan.

Fusion of the two stocks is going on to-day in Fiji. With the object of learning what the species formula of the autochthonous Fiji population was, the remote and rather inaccessible village of Nasoqo in the mountains at the head waters of the Rewa River was visited and a group of 15 persons treated.

Among 546 hookworms obtained not a single *A. duodenale* was encountered, the ancylostome index being nil (a few *A. ceylanicum* were found as in the mountain villages of Java but these are derived from dogs and have no bearing on the *A. duodenale* index).

The worm counts made on the town dwelling Fijians (village of Nausori) confirm the findings at Nasoqo, but, as we have seen, they show evidences of contaminative infection by *A. duodenale* from North Indian sources, that is, from plantation and factory coolies working and living in the same village.

Until further and more detailed survey work is done in Polynesia we may assume that the primitive ancylostome index in this region is nil.

This observation at Nasoqo is of the greatest value, for it was made on a pure uncontaminated population of two fused South Sea ethnic stocks. Chinese, Japanese, Portuguese and East Indian immigrations have been for several years altering the primitive index of South Sea Islanders in the towns and villages near plantations.

The absence of *A. duodenale* in Fiji among uncontaminated autochthones indicates that races carrying *A. duodenale* as the Egyptians, Chinese, Burmese, Japanese and North Indians have never colonized there, and it shows that the Fijians, wherever their stock originally came from, did not come from north of 20° N. latitude and have never been in contact with people from those latitudes.

This is a matter of considerable interest in view of the probably erroneous opinion held by some that the Polynesians originated in northern Africa or Asia and that during their migrations southward they sojourned in Fiji.

Additional surveys are urgently needed in Polynesia before we can speak confidently in this matter.

To one who has been intimately acquainted with Malays and Javanese and has seen something of the Papuans, Solomon Islanders, Fijians, Tongans, Samoans and Maoris there is beyond certain linguistic similarities nothing to

suggest Malayan immigration to Polynesia, and these similarities may otherwise be explained.

FURTHER OBSERVATIONS AND CONCLUSIONS.

The hookworms harboured by a people depend on geographical, racial and climatic conditions and circumstances.

A people can only harbour the worm species which have been existing as embryos in the soil of their immediate environment.

The hookworms harboured by a person may disclose the influences to which he has been subjected in another environment.

The relative number and species of hookworms will sometimes furnish indications as to the ethnic origin of a people about whose history there is no record.

There has been a migration of rather large numbers of Asiatic people into parts of Indonesia.

These people came from north of 20° N. latitude, probably India or Burmah, and colonized in fairly large numbers in mid and East Java, Bali, Timor, Lombok and Madura as is evidenced by the hookworm species formula of the people now resident there. Other parts of Indonesia may have been visited as no doubt they were, but not by people in sufficiently large numbers to affect appreciably the normal formula of Indonesian people.

This Asiatic emigration did not extend to Polynesia, Fiji and Tonga for there are no helminthological evidences of it.

Whatever migration to the South Seas occurred, if any, it must have taken place by people from south of 20° N. latitude, that is, from Madras, or Malay Peninsula, Sumatra or other parts of Indonesia or previous to the Asiatic colonization of Java and Bali. That is to say by a people with an ancylostome index amounting to nil.

The presence of pure cultures or of relatively pure cultures of *Necator* in natives of Asia or Africa indicates that their hosts belong in the south rather than to the north, for the primitive distribution of *Agchylostoma duodenale* seems to have been limited to regions north of 20° N. latitude, while *N. americanus* was distributed south of this line.

A. duodenale may have been conveyed to or from the Orient along the old trade routes. But there are no evidences that *Necator* was introduced into Southern Europe from the east or into Egypt from South Africa for *Necator* is not found in Southern Europe or in Egypt. At any rate this species does not seem to be distributed to the latter places.

Careful surveys are necessary here as elsewhere, for it must be remembered that *N. americanus* was for years overlooked in India by British, Dutch and French doctors, although the species exists there in enormous numbers. Practically every East Indian over 14 years of age living north of Calcutta being infected with both species.

If certain tribes in America are found to be infected with *A. duodenale* as

well as *Necator* this will suggest their having come to this continent by way of the sea from those countries in Asia where *A. duodenale* and *Necator* are found to be infecting the natives, *i.e.* Japan and China. -

A careful hookworm survey of existing Indian tribes may disclose the presence of more than one primitive stock. In other words: (a) a stock free from hookworms derived from Asiatic trans-Behring ancestors, (b) a stock harbouring both *A. duodenale* and *Necator* derived from Asiatic trans-Pacific ancestors, and (c) a stock with a pure *Necator* infection derived from Polynesian or Indonesian trans-Pacific ancestors.

If the Indian races or some of them living in regions unvisited by strangers are found to be free from hookworms this would furnish some grounds for supposing that the races in question had been derived from ancestors who had crossed from Asia by way of Behring Straits.

Surveys of all ethnic groups of people are urgently required before there occurs that mixing of stocks which will efface the individualities of species formulas.

Surveys are particularly desired in America among various North, South and Central American tribes, in Asia (subtropical), the Philippines, in Melanesia, Micronesia, Polynesia, Australia and in Madagascar and Easter Island.

THE ANOPHELINE WATERS OF SOUTHERN FLANDERS.

BEING A REPORT ON THE AREA OCCUPIED BY THE
BRITISH SECOND ARMY IN FRANCE.

BY A. D. PEACOCK, M.Sc., CAPTAIN R.A.M.C. (T.F.).

(With 1 Map and 1 Text-figure.)

UNDER instructions from Colonel W. W. O. Beveridge, C.B., A.D.M.S. (Sanitation), G.H.Q. France, a survey of the British Second Army Area in Southern Flanders was made in order to ascertain the condition of that region in regard to Anopheline mosquitoes. Permission to publish an account of the work has kindly been accorded by Lieutenant-General Godwin, Director-General of Medical Services.

A certain amount of similar work had already been performed by the writer in 1915 under instructions from Lt.-Col. J. Rutherford, A.D.M.S., 50th Division and also, in 1916, under instructions from Lt.-Col. H. Barrow, D.A.D.M.S. (Sanitation), Second Army, near the southern borders of the Second Army Area, and the results went to suggest that Anophelines would be found widely distributed and that, in certain areas at least, about one-fifth of the number of waters examined would harbour Anopheline larvae.

The first possibility was supported by the investigations in England of Nuttall, Cobbett and Strangeways-Pigg¹ and the later surveys of Grove, Parsons and Macdonald², in which it was ascertained that Anophelines could be found in all parts of England and Wales. The second possibility remained to be proved by further enquiry.

Regarding the species of Anophelines likely to be found, the late Prof. R. Blanchard, of the École de Médecine, in July 1918, informed the writer that he had records showing that Anophelines were generally distributed in the areas occupied by the French Armies, that is, in the country south-west of that in English occupation, the most common species being, as in England, *A. maculipennis*, while *A. bifurcatus* was less common and *A. plumbeus* (*nigripes*) had been but seldom found. Careful search for the last-mentioned however still remained to be performed.

¹ Studies in relation to Malaria, *Journal of Hygiene*, vol. 1, 1901.

² Reports and Papers on Malaria contracted in England in 1917. *Reports to the Local Government Board on Public Health and Medical Subjects* (New Series, No. 119).

The data of this report have been derived from three sources:

- (1) From surveys made by the writer during the summers of 1915 and 1916.
- (2) From a survey made by the writer during September, 1918.
- (3) From various collectors, the bulk of whose specimens had been collected during September, 1918, these specimens being identified by the writer.

SCOPE AND SCHEME OF WORK.

The main enquiry was commenced on August 30, 1918, and continued during the month of September, other duties preventing earlier and later investigations.

In view of the brief period of time available and the lateness of the season it was decided that the enquiry should be concerned with ascertaining, firstly and most importantly, how widely spread were Anophelines in areas most frequented by troops; and, secondly, if opportunity permitted, how high was the degree of infestation of any area or areas. In other words it was proposed to make a general survey and then, if possible, at least one detailed local survey.

As promising the speediest and most profitable return of information the plan followed was that of searching for the Anopheline breeding waters. The search for the haunts of the adults, involving laborious work among the dark places of rooms, cowsheds, stables and latrines, was regarded as too time-consuming and was not pursued. Again, except in a few instances, experiments in rearing larvae and pupae obtained from Anopheline and suspected waters, in order to determine the genus of the mosquitoes and the species of the Anophelines, were impracticable as also was the identification of the species of the Anopheline larvae captured.

The measure of success attained has depended a great deal upon the generous and ready help rendered by various sanitary officers and their non-commissioned officers and men. Intelligent work in searching for Anopheline waters was performed by these non-commissioned officers and men, who, from their civilian callings, were well fitted to assist in the investigation.

The co-operation of officers of the medical service was also invited, an illustrated circular and a pro-forma being distributed, the circular indicating the objects of the survey and detailing the methods of collection and despatch of specimens and information, while the pro-forma was designed to facilitate the forwarding of such specimens and information. These were distributed from the Office of the Director of Medical Services, Second Army, to all Deputy and Assistant Directors of Medical Services, Officers Commanding Stationary Hospitals, Casualty Clearing Stations, Mobile Laboratories and Sanitary Sections. In certain cases the A.D's.M.S. repeated the circular to Officers Commanding Field Ambulances and to Regimental Medical Officers. All the assistance rendered is acknowledged later.

TECHNIQUE AND METHODS.

The apparatus employed for dipping was the top of an infantry mess-tin, the inside of which was painted white to facilitate detection of the larvae. The handle was lashed to a stick about 3 feet long in such a way that the tin could be manœuvred into three positions (1) in the same straight line as the stick, (2) at an angle of 45° or (3) at right angles. With the co-operation of Lance-Corporal Hicks, 71st Sanitary Section, a more perfected little device was made and is here illustrated.

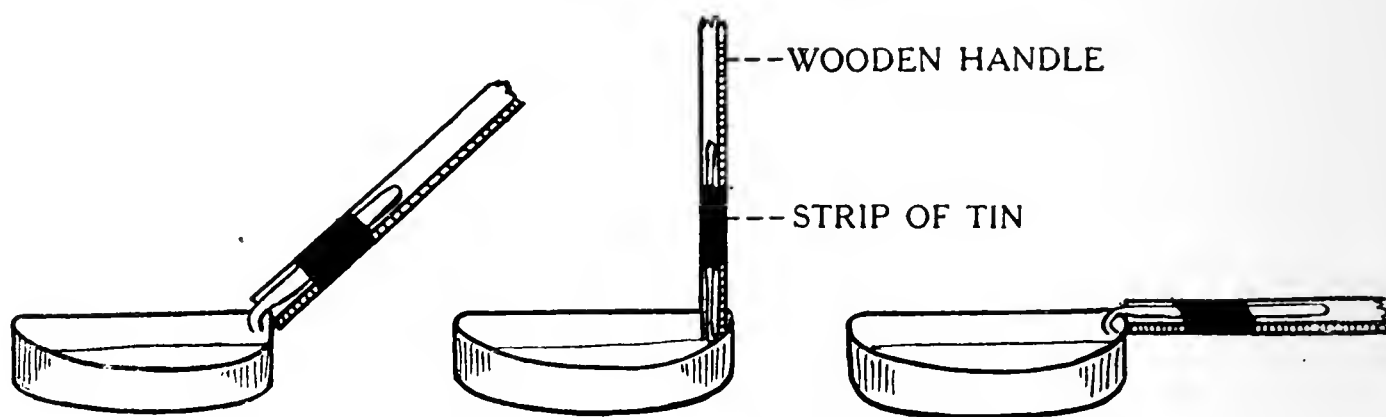


Fig. 1. Diagrams illustrating the device used in collecting larvae from water.

The strip of tin was tacked around the flat wooden handle so that a slot was formed for the insertion of the mess-tin handle. By altering the position of the mess-tin handle in the slot the device could be used rigid in the three different positions indicated.

The position of the dipper could be altered at convenience according to the character of the vegetation and banks of the water under examination, the third position being useful when skimming and to obtain specimens well away from the margin, and the second when the banks of the breeding water were high. Anopheline larvae were obtained by merely lowering the dipper into a pool or by scooping up the water but many methods had to be employed. It happened not infrequently that, while repeated scooping yielded no result, long skimmings of the surface among the vegetation were successful. In certain cases, when other methods failed, skimming the fringes of algae proved successful as did dippings taken at greater depths than the superficial two inches or thereabouts. No note in the negative was made in regard to a pool which looked at all promising for Anophelines till about 20 dippings proved fruitless.

Observations were recorded in a notebook on the spot.

THE ANOPHELINE CONDITIONS IN VARIOUS DISTRICTS.

The area bounded by imaginary lines joining the most outlying places visited, viz. Millam, Wylder, Woesten, Ypres, Kemmel, Armentières, Arques and Moule, is approximately 350 square miles, but the area examined with varying thoroughness by all workers amounted to about 120 square miles only. Most of the districts of the complete area, however, have been touched and those described may be regarded as representative. Areas densely popu-

lated by soldiers and likely to remain so, naturally received the most attention. The conditions existing in 8 main districts and 12 isolated districts of the army area are described and certain observations from two other parts of the British military zone are also recorded. The 8 main districts have been named as follows: Advanced, Poperinghe, Hazebrouck, Southern, Rubrouck, Central, Waterlands and Armentières. In addition, observations were made in the vicinity of the following places: Houtkerque; Herzeele, Wormhoudt, Esquelbec and Zeggers Cappel; Arneke and Ledringhem; Steenvoorde, St Sylvestre Cappel, Cassel, Winnezeele and Abeele; La Motte; Ebbilinghem; Wallon Cappel; Steenwerck; La Crêche; Le Romarin; Arques, Blendecques, Longpont and Mal Assise; Nielles lez Blequins; Clerques. The two districts outside the army area were Serqueux and Abbeville.

The general features of each district may now be described.

Advanced District. For military reasons this district could only be surveyed generally and certain special spots were selected as suitable places for search. The district is rectangular, 8 by $2\frac{1}{2}$ miles, with an area of 20 square miles and occupied the eastern side of the army zone. It had suffered severely from shell fire and the sites examined were much pitted with shell-holes. The number of sites examined was at least 220 but only 12 were found infested with Anophelines. In 8 cases larvae were found and in 3 the adult fly was taken near human habitations. Of the 12th there are no details. Of these 8 breeding waters, 5 were shell-holes, 2 were pools and 1 a stream. Of the habitations, 2 were medical aid-posts and 1 a billet. In 2 cases only were the larvae numerous. One observation from Ypres is important from the point of view of the number of Anophelines found. Out of 31 mosquitoes caught indiscriminately in an aid-post, 23 were *Anopheles maculipennis* ♀♀. At 2 centres, Dickebusch and Reninghelst, notwithstanding careful searching in many likely waters, negative results were obtained. The small number of infected waters is noteworthy and is difficult to explain. The character of the region in more quiet times did not appear different from the other regions which show a much higher proportion of Anopheline waters and the manner of search was the same as usually employed, so it is conceivable that the sparsity of infested waters may be attributed either to the lateness of the season or to the effect of poison gas used in fighting over this region or to both factors.

Poperinghe District. This irregular district has an area of about 40 square miles. It is mostly level farm land and is distinguished from most of the other districts described by the number of small woods it possesses. The method of search employed was to make a detailed survey of the land in the vicinity of the roads radiating for about 3–5 miles from Poperinghe and traversing the country to the villages Woesten, Elverdinghe, Vlamertinghe, Abeele, Watou and Proven, as well as of two other small regions (totalling 7 square miles), one in the neighbourhood of Hamhoek and La Lovie and the other

near Abeele and-Hamhoek. Altogether about 12 square miles were inspected. Approximately 200 waters were examined, most of them farm pools, though streams, canals and shell-holes have also come under survey, and, in number and character, it is considered that this sample is representative of the district. Twenty-nine Anopheline waters were found, in 20 cases harbouring few larvae and in 8 harbouring many. One record is incomplete. That is, 14·5 per cent. of the waters examined contained Anophelines or about 1 pool in every 7, an average, also, of about 2·4 per square mile.

Hazebrouck District. This district is small, approximately 3–4 square miles, and is composed of open farm land. It contains the town of Hazebrouck and the village of Hondegheem. It was searched almost exhaustively and the results of the enquiry may be regarded as giving a correct idea of its condition in regard to Anopheline distribution. The number of waters was 114, practically all pools, of which 16 contained Anophelines, 9 with few, 4 with many and 3 with a number undetermined. The proportion of infested waters therefore was 14 %, or approximately 1 pool in every 7, an average of about 5 per square mile.

The results of the more general survey of the region north of the Hazebrouck district showed 7 infested pools out of the 53 investigated, that is, 13·2 % or about 1 in every 7 to 8 pools examined.

Southern District. This district, which is similar in character to the Hazebrouck district, was worked in detail for pools in the summer of 1916. It is irregular, about 20 square miles in area, the chief villages being Borre, Caestre, Pradelles, Strazeele, Fletre, Merris, Meteren and Neuve Eglise, with Bailleul as the important town. The only exact records preserved relative to this district are those concerning the location of the Anopheline centres and consequently the proportion of infested waters cannot be given. A general note exists which states that approximately 20 % were Anopheline but in view of the recent findings for the Hazebrouck district the statement cannot be accepted as final. As, however, the number of infested waters discovered was 64 it can be stated that there were 3 Anopheline waters per square mile.

Rubrouck District. This district, about 3 square miles in area, consists of open level farm country and the villages around which the survey extended were Rubrouck and Broxeele. The survey was a fairly detailed one but, being made by assistants, it is probable that certain infested waters were overlooked owing to the lack of training of these workers. Out of approximately 183 waters examined, 10 were found to be Anopheline, that is, 5·5 %, or 1 pool in 18, an average of 3 per square mile. The number of larvae was not noted by the observers.

Central District. This district of about 5 square miles includes the high-lying villages of Godewaersvelde, Boeschepe and Berthen and the land sloping away from them. The altitude rises abruptly to 200 feet above the sea level.

The waters examined lay in the vicinity of the roads intersecting the district and numbered 12, and were mostly pools. The comparatively small number is due to the fact that the waters appeared to be less numerous than in the surrounding lower-lying country. No Anopheline waters were discovered but in view of the small number of sites examined it cannot be asserted that no Anophelines existed.

Waterlands District. The waterlands of this part of Flanders are widespread and low-lying and are characterised by the presence of many canals which intersect them and the network of ditches of varying widths and depths. In the Second Army area the waterland region is irregular and not of great extent, being about 15–20 square miles. It penetrates the north-west boundaries of the army zone and is almost detached from the main waterlands to the north, the junction between them being narrow and running between high land on either side. The regions surveyed were part of the south-west fringe of the main waterlands, from Millam and Watten, about $1\frac{1}{2}$ miles and the area bounded by imaginary lines joining Watten, Moulle, St Omer and St Momelin. The latter region is about 10 square miles in extent and is bounded on the east by the canal between Watten and St Omer, which receives all the drainage from the western network of streams and ditches. Particular attention was directed to pools in the vicinity of Moulle on the west, pools and ditches near Bleue Maison and Watten in the north, pools near St Momelin on the east and the main canal and a ditch running roughly north and south between Watten and St Omer. To the north, waters round the villages of Wylder and Rexpoede were investigated by Lance-Corporal Newman, R.A.M.C.

Near the village of Millam 4 Anopheline pools were discovered, 2 containing numerous larvae and 2 with few. The long ditch running by the side of the road from Millam to Watten was typical of the district. In width it varied from 4 to 8 feet and in depth to about 4. It received the drainage from smaller ditches on the eastern slopes. It was very rich in flora and the greater proportion of it was covered with water vegetation, open patches of water being exceptional. The predominant water weeds were marestalk (*Hippuris*), starwort (*Callitriche*), duckweed (*Lemna*) and fringed water-lily (*Limnanthemum*) while water-cress (*Nasturtium*) and algae (*Spirogyra* mostly) were also present in places but, compared with the other plants, were small in quantity. Marginal rushes were also found but the banks were mostly grassy. The larger fauna consisted of eels (*Anguilla*), sticklebacks (*Gasterosteus*), fresh-water shrimps (*Gammarus*) and water hog-lice (*Asellus*). Examinations were made in at least 7 places along this ditch, many dippings being made at each examination but Anophelines were found in only two places and in small numbers. The first capture of 3 small larvae was made by repeatedly skimming the water above the algae and fringed water-lily, the second capture, 5 small larvae, by the same method above algae.

In the large district the following results were obtained. At Moulle three extensive sheets of water were examined. One, a large lake near the chateau, had a great deal of marginal duckweed and algae while the central water was more open but supported large floating mats of algae. The Anopheline larvae were of all sizes, extremely numerous and could readily be seen dotting the surface of the water. *This is one of the two waters which have been discovered to be highly infested and by reason of this and their large extent constitute real menaces.* Another small lake near by and of similar character, in spite of repeated search, yielded very few larvae, while a third gave a negative result. At Bleue Maison a few Anopheline larvae were found in a ditch overgrown with vegetation consisting mostly of fringed water-lily and tall sedges. At Watten two long ditches, each about 300 yards in length, and characteristic of the district, were examined intensively. They were mostly covered with duckweed, fringed with high rushes and had occasional open patches of water. No Anophelines were found. Another ditch running by the western side of the Watten—St Omer road, was also examined in detail. It was choked with high rushes and grasses and the surface was covered in many places with fringed water-lily and duckweed. The result was negative. The main canal was fringed in places with duckweed and grasses and stranded stalks of various plants but the results of many examinations were also negative. On the outskirts of St Momelin a small pool was discovered slightly infested. At Moulle, where the highly infested lake was situated, there was a dysentery camp for Chinese and a large Casualty Clearing Station—a dangerous association.

Twelve waters which included pools and ditches in the Wylder—Rexpoede district gave a negative result.

Armentières District. This district is of interest as showing, in contrast with most of the areas surveyed, the Anopheline conditions existing near a large town. In extent it is about 3 square miles and lies between Armentières and its outlying suburb Nieppe. About two-thirds is open, low-lying farming country. The examination was made in the summers of 1915 and 1916 and in the town showed negative results with regard to the infestation of receptacles such as water butts, ornamental pools and reservoirs. The canal also was negative. On the northern outskirts near the canal was a patch of low-lying, grassy, water-logged land which yielded numerous larvae. From the south-eastern suburb, Chapelle d'Armentières, one specimen of *A. maculipennis*, female, was brought to the writer. A mile to the east of the town a small Anopheline pool was discovered but no record of its degree of infestation has been retained.

Isolated Districts. The results obtained in the isolated districts examined may be given conveniently in tabular form.

District

Notes on Examination

Houtkerque; 1½ square miles.	About 18 waters examined; 1 Anopheline pool found; number of larvae not stated but probably few.
Herzeele; 1½ miles of road.	About 6 waters examined; result negative.
Wormhoudt, Esquelbee, Zeggers Cappel Road; about 4 miles.	About 15 waters examined; 1 Anopheline pool found.
Volkerinckhove; ½ square mile.	Nine waters examined; 2 Anopheline pools found; few larvae.
Arneke, Ledringhem; about ¾ square mile.	About 8 waters examined, 5 proving Anopheline; 3 being ditches, 1 a small marsh, 1 a pond; 4 contained few larvae. 1 (the marsh) had many.
Steenvoorde; roads radiating to St Sylvestre Cappel, Cassel, Winnezele and Abeele; from 3-5 miles of each road taken.	About 30 waters examined, 3 proving Anopheline. A pool near Steenvoorde contained many larvae, a bomb crater in the same vicinity contained few, while from a pool in the town of Cassel a single larva was forwarded.
Ouderzeele; about 3 square miles.	About 20 waters examined with negative results.
La Motte.	Village surrounded by large woods; in and near the village 6 waters examined comprising 3 parts of the Canal de Nieppe, one lake, one pond and one ditch; 3 were Anopheline, one part of the canal and the lake yielding each 1 larva and another part of the canal 2 larvae; on the Hazebrouek Road 4 other waters were inspected, 3 ponds and the stream Bras de la Bourre; several were found at the margins of the stream, and in one pond they were numerous.
Ebblinghem.	Five waters examined showing one pond with a few larvae.
Wallon Cappel.	Eleven waters examined, yielding 3 ponds infested, 2 with few and 1 with numerous larvae.
Steenwerek.	No details.
La Crêche.	Anopheline pool; larvae numerous.
Le Romarin.	Anopheline pool; larvae few.
Arques, Blendeeques, Longpont, Mal Assise.	About 8 waters examined yielding 4 infested; one was a ditch, the second a pool—both with few larvae, the third, a ditch, contained an extremely large number and in the fourth, a pool, they were numerous. Adult Anophelines (<i>maculipennis</i>) were found in Blendeeques.
Nielles lez Blequins.	Few larvae found in a pool.
Clerques.	Three waters in vicinity examined; one Anopheline larva in one pool.

Notes on Anopheline districts in France outside the Second Army Area.

Anopheline larvae were found among a sample of many hundreds of Culicine larvae sent by Capt. Wadsworth, R.A.M.C., from Serqueux. The pond from which they were obtained was in close proximity to a malarial segregation hospital. The specimens were collected on August 22nd, 1918. At Abbeville between August 22nd and 25th, 1918, were found 3 large Anopheline ponds and one water channel in the Faubourg Thuison, one of the ponds containing numerous larvae and the other two containing few, while the channel also yielded few. Larvae were also found in the marginal vegetation of a stream running through the Triage (goods station) and at Mautort, some 3 miles outside Abbeville, 3 large Anopheline pools were discovered, one with numerous larvae and 2 with few.

GENERAL NOTES ON THE ADULT AND LARVAL ANOPHELINES FOUND.

At various times during 1915, 1916 and 1918 about 40 adult Anophelines of this area have been examined by the writer and all proved to be *A. maculipennis*. Of this number 27 were captured wild and the remainder reared from larvae or pupae. Only two of these were males, both being hatched in captivity. In addition Father Legros, R.C., C.F., informs me that specimens which he collected alive at Moulle and which were identified for him at the British Museum proved to be of the same species. No records or specimens of *bifurcatus* or *plumbeus* (*nigripes*) have come to the writer's notice in this region.

A certain amount of interest is attached to the capture by Capt. Rankin, R.A.M.C., of 23 *maculipennis*, all females, out of a total catch of 31 mosquitoes obtained in an afternoon from the inner gas curtain of a dark, damp aid-post. No discrimination was exercised in taking the specimens. This is the only relatively large catch of adults which has come to the writer's notice in Flanders, all other catches yielding usually one, and not more than two, specimens.

The proportion of Anophelines to Culicines cannot be stated even approximately but judging from general observations on larvae and adults it must be very small.

As regards the larvae captured during September 1918, the only remark that may be made is that no seasonal preponderance of any one size of larvae could be detected.

NUMBER, NATURE AND CHARACTER OF ANOPHELINE WATERS
DISCOVERED.

The total number of examinations made was at least 1233, all except 12 relating to waters, these 12 exceptions referring to adult specimens captured. The exact number of examinations is uncertain as certain collectors forwarded approximations only of the number of waters coming under their survey. Of the total number of examinations, about 558 were made by the writer—at least 210 in the summers of 1915 and 1916, and 348 during the month of September, 1918—and 675 by the various collectors. Out of these 1233 records, 178 show the location of Anopheline haunts. The proportion of Culicine waters cannot be estimated as collectors have not furnished full enough details, most contenting themselves with noting the Anopheline waters only. Of the 178 Anopheline haunts 133 were discovered by the writer, 67 in 1915 and 1916 and 66 in 1918, and 45 by collectors, 5 in 1915 and 1916 and 40 in 1918. Only 5 of the Anopheline records refer to adults. The nature of the 173 Anopheline waters is given in the following table.

Analysis of the types of 173 Anopheline waters discovered.

Pools, etc.	127 (73.4 %)	Pools, 121; lakes, 2; moats, 4.
Holes	16 (9.25 %)	Shell-holes, 8; pits, 8.
Streams, etc.	12 (6.9 %)	Streams, 9; canals, 3.
Ditches	8 (4.6 %)	Includes one small stream in which water was very low and stagnant.
Marsh, etc.	6 (3.5 %)	Marshes, 5; water-logged field, 1.
Receptacles	2 (1.2 %)	Tin*, 1; concrete basin, 1.
No details	2 (1.2 %)	

* On another occasion, numerous larvae were found in a petrol tin, cut to make an ablution basin, stranded in the mud at the side of an Anopheline pool.

The term "pools" refers principally to waters found in the farm-lands and for the most part lying in clayey soil. Many of these are natural but many undoubtedly are really waterholes of artificial origin having been dug in years past by farmers for use by cattle. These waterholes are usually circular, comparatively small, varying from about 20-60 feet in diameter, and are usually surrounded by pollard-willows. Their vegetation varies, some being clear of weeds, others with marginal weeds and algae more or less loosely distributed while others again have a dense mat of duckweed completely hiding the water. Moats with vegetation similar to that of pools are found round many of the farms and chateaux. The term "canal" is used in the strict sense of the word. In one case the map refers to certain water as a canal when the water really takes on the character of a narrow stream running fairly rapidly. This has been classified as a stream.

The character of the Anopheline waters in regard to possible pollution from various sources has been noted nine times; on four occasions the water being described as "dirty"; once as "tea-coloured," the reason for such a colour being unknown; once as "greenish with numerous larvae," the green possibly due to cow manure and not to confervoids; once as "brown, not a likely place"; once as "poor quality, unusual place"; once as "containing some pollution." In one case where the water was dirty the circumstances were exceptionally interesting. The pool in question was circular and about 60 feet in diameter, with many high sedges and a certain amount of duckweed at the margin. The water was black and turbid. Into one region ran the effluent of the inefficient soakage pit of a hospital bath-room, the effluent water being still soapy. Opposite this the pool drained into a narrow channel running through a culvert. Altogether a most unlikely spot for Anophelines! But near the entrance to the culvert both Anopheline and Culicine larvae were taken. The Anophelines did not show any unusual features but the Culicines presented a remarkable appearance as if they were covered with a white fungoid-like growth. This was probably due to the soap suds from the effluent. In another instance larvae were found in a small marginal patch of water surrounded by the marginal grass of a pool reddened by the rust of ration

tins which had been thrown into it. Ten waters, rendered disturbed or turbid through rain, also contained Anopheline larvae.

The floral and faunal associations of the Anopheline larvae.

(a) *Floral*. Out of 106 records the following analysis results:

Flora										Number of times during which Anopheline larvae were found associated
Grass	18 (17 %)
Grass predominating, with the addition of confervoids and the common waterweeds	17 (16 %)
Algae—mostly <i>Spirogyra</i>	18 (17 %)
Algae—with addition of broad-leaved pond-weed (<i>Potamogeton</i>), water-cress (<i>Nasturtium</i>), fringed water-lily (<i>Limnanthemum</i>), duckweed (<i>Lemna</i>), flag (<i>Iris</i>)	11 (10·3 %)
Water-cress	6 (5·6 %)
Water-cress predominating, with addition of other vegetation	2
Sedges (<i>Carex</i>) and other vegetation	2
Confervoids	2
Broad-leaved pond-weed and duckweed	2
Rushes (<i>Juncaceae</i>) and algae. Rushes and maretail (<i>Hippuris</i>). Lily and algae. Fringed water-lily and sedges. Flag. Flag and duckweed. Weed unidentified	Each	1

On six occasions larvae were found in water with no visible foodstuffs. Fifteen records are incomplete. Waters in which the visible vegetation was grass or algae or predominately either of these gave positive results almost certainly. Association with water-cress was not infrequent. In ponds covered entirely with a dense mat of duckweed Anophelines have never been found but at the margins of certain pools of this character, in open patches of water among tall fringing vegetation, the larvae have been taken. The low degree of infestation of the ditches of the waterlands is interesting.

(b) *Faunal*. The results of 167 records show the following:

Fauna										Number of times during which Anopheline larvae were found associated
Culicine larvae alone	40 (24 % nearly)
Water hog-louse (<i>Asellus</i>) alone	11 (6·5 %)
Fresh-water shrimp (<i>Gammarus</i>) alone	2
Culicines and water hog-lice	5
Culicines, water hog-lice and fresh-water shrimps	1
Water hog-lice and fresh-water shrimps	1

The number of times during which Anopheline larvae were found alone was 109 (65 % approximately). Fresh-water fish of many kinds have also been found along with Anophelines. Water-boatmen (*Notonecta*) have been observed, in an aquarium kept at a Casualty Clearing Station, to prey upon and suck Anopheline larvae.

The conclusions, therefore, are that waters with a vegetation consisting of grass or algae or with vegetation in which grass or any alga is predominant are almost entirely Anopheline and that all waters should be regarded as suspected Anopheline habitats.

The Degree of Infestation of Anopheline Waters. In regard to the number of larvae found in the waters it has been found necessary to establish a standard by which to estimate the degree of infestation of such waters.

While the number of dips taken at a water varied with the conditions discovered, the result of usually 20 dips per water, taken at various parts, was judged to indicate the degree of infestation, and, in practice, it was found that the catches yielded extremes, there being captured either few or many. The term "few" refers to *total* catches up to 10 or thereabouts and the term "numerous," interpreted rather broadly, to those in which the average yield *per dip* was 3 and over.

The number of records is 106, from which it is found that the number of waters with few larvae was 67 (63.2 %), those with numerous larvae 22 (20.7 %), while 17 (16.1 %) records are doubtful.

It was impracticable to ascertain the Anopheline population of any water but it may be recalled that in two instances, at Moulle and Arques, it would run into many thousands.

The Degree of Anopheline Infestation of the Second Army Area. In estimating the significance of these results regard must be taken of the comparatively few pools examined in certain regions, the inexperience of some of the collectors and the lateness of the season. These factors would tend to produce results which would indicate that the number of Anopheline waters and the degree of infestation of the districts were lower than those actually obtaining.

The weather conditions during the survey, on the whole, were good for the season of the year, there being a number of hot days. For about seven days the weather was wet, bleak and cold but it is unlikely that it influenced the results to any great extent.

In estimating, from the data discovered, the Anopheline conditions existing in the Second Army area three questions must be answered, viz.:

- (1) What is the geographical distribution of Anopheline waters?
- (2) What proportion of waters is infested with Anophelines?
- (3) What is the degree of infestation of these waters?

In regard to (1) it can be stated that Anophelines are generally distributed throughout the Second Army area. Out of 20 neighbourhoods examined, only in 4, Herzelee, Ouderzelee, Steenvoorde (south and west) and Gode-waersvelde, Berthen and Boeschepe (high land), were no Anophelines found, but it is quite probable that more careful search would yield positive results. It may also be added that the greater number of Anopheline waters were in close proximity to billets.

In answering the questions (2) and (3) it would be incorrect to total the

findings for all the districts and then strike averages because the different districts vary in their characters and have been surveyed with varying thoroughness and skill. It seems fairest, therefore, to take the results obtained in a district which, in its physical features, is representative of the greater part of the Second Army area and which has been carefully and thoroughly surveyed. Having regard then, to the season of the year when the investigation was made, the findings of the Hazebrouck district are selected as representative. The findings are:

Proportion of waters infested with Anophelines	14 % about 1 in 7
Number of Anopheline waters per square mile	5
Proportion of Anopheline waters with numerous larvae	25.0 %
" " " few " 	56.3 %
" " " undetermined number of larvae	18.7 %

As a matter of interest the findings for the Poperinghe and Southern districts, which were surveyed in fair detail, and the whole Second Army area, as well as the findings for certain eastern counties of England are given in the following table. The figures relating to England have been obtained from the writer's analyses of the data of 64 waters given by Nuttall, Cobbett and Strangeways-Pigg¹. Similar figures to these with regard to the

Table showing comparison between the degrees of infestations of various districts in Flanders and England.

Particulars	Hazebrouck District: Standard	Poperinghe	Southern	Whole Second Army area	Eastern counties* of England, July and September, 1900
Percentage of waters infested with Anophelines	14.0 %	14.5 %	20 % (?)	14.0 %	—
Number of Anopheline waters per square mile	5	2.4	3.2	1.4	—
Percentage of Anopheline waters with numerous larvae	25.0	27.5	?	20.7	56.25†
Percentage of Anopheline waters with few larvae	56.25	69.0	?	63.2	29.7
Percentage of Anopheline waters with doubtful number of larvae	18.75	3.5	?	16.1	14.0

* Counties of Lincolnshire, Norfolk, Suffolk, Cambridgeshire, Huntingdonshire, Bedfordshire, Hertfordshire, Essex and Kent.

† 48.4 % numerous, 7.85 % fairly numerous.

later finds of Grove, Parsons and Macdonald² in England cannot be made from their reports. This is unfortunate as these investigators have surveyed districts where cases of malaria have recently occurred among troops and civilians who have never been out of the British Isles.

It will be noticed that the proportion of English Anopheline waters which contained numerous larvae was much greater than that of the Flemish waters.

How the degree of infestation of the Flanders region would compare with other areas in European countries in the temperate zone cannot be given, as

¹ *Loc. cit.*

² *Loc. cit.*

the subject, to the writer's knowledge, has not received sufficient attention. The evidence given above, however, would seem to indicate that, from an absolute standard, the degree of infestation for the Flanders region is low.

Comparisons can only be made then from existing standards which are all concerned with conditions existing in sub-tropical and tropical countries. To illustrate, a few examples may be cited from countries in the various theatres of war. In Italy, in 1900, Grassi caught 200 adult *Anopheles* during a two hours' coach ride across the plains of Capaccio¹; in Macedonia "the quantity of *Anopheles* reaches absolutely extraordinary proportions in some regions, it being possible to catch hundreds of dangerous mosquitoes in a few hours²"; in Palestine, I am informed by Capt. Adams, R.A.M.C., he found 30 % of the wells etc. in Jaffa to be Anopheline. From the number of larvae found in Flanders and, bearing in mind that only a proportion of larvae reach maturity, it may therefore be safely asserted that the degree of Anopheline infestation of Flanders is very low compared with that of these sub-tropical malarious countries.

THE PROBABILITIES OF THE SPREAD OF MALARIA AMONG TROOPS IN SOUTHERN FLANDERS.

In estimating the probabilities of an outbreak of malaria among the troops in the area surveyed two other considerations had to be taken into account:

- (1) How many troops and civilians were malarial subjects.
- (2) What had been the incidence of "primary" malaria among troops from non-malarial districts of the British Isles.

Owing to the insufficiency of the epidemiological evidence it is impossible to discuss these in anything but a general way. Undoubtedly many divisions of troops which occupied the area contained a high proportion of malarial subjects as, for instance, the Indian Division which occupied the area as early as 1914-1915, and a succession of colonial troops, old and new army divisions which had served in the Eastern Fronts, as well as Chinese and coloured labour corps. My only information as to the incidence of malaria among civilians is meagre and was received from Major McNee, D.S.O., R.A.M.C., who stated that he had been informed by a local general practitioner that an outbreak of malaria had occurred about 1905 at Steenwerck. However, from the military evidence alone it is certain that the district has never had such a large and varied population of human malarial hosts, even during the conditions which may have existed in the many campaigns conducted over this historic fighting ground.

At the time of writing an insignificant number of troops had contracted "primary" malaria.

¹ Cited by Nuttall, Cobbett and Strangeways-Pigg (1901).

² Armand-Delille, Abrami Paiseau and Lemaire. *Malaria in Macedonia*, Military Medical Manuals, 1918.

All factors considered, then, it would seem that there was little likelihood of an epidemic of malaria occurring in the British Army in Flanders. Still less likely is an outbreak to-day among the civilian population. Further, as will be shown presently, even in the event of an outbreak, the entomological conditions are easily amenable to control.

NOTES ON CERTAIN PREVENTIVE MEASURES.

The object of this section is merely to indicate certain conditions which were peculiar to the existing circumstances and which would have demanded special consideration in the event of the institution of prophylactic measures.

Military exigencies permitting, a great deal of most useful preventive work would have been easily practicable owing to the type of Anopheline waters existing in this zone. Most of these are pools of comparatively small size and thus amenable to treatment by the usual methods of dragging them free of weeds and oiling. If all such habitats were treated successfully the Anophelines of the area would be brought under control.

In the case of certain pools important considerations would arise when deciding what action should be taken when the Anopheline water was (1) the sole source of supply for military horses, (2) the sole source of supply for civilians or their farm stock.

The first difficulty could be overcome in certain cases by digging a separate small water-hole at the side of the pool, and then periodically treating the pool. In this way water could be obtained which would not offend the sense of smell of horses. In many cases a water-hole would be unnecessary as many transport units are provided with pumps and hose which permit the withdrawal of water from below the surface.

In the second instance arrangements would have to be made with civilians, through the usual authorities, that their water should be treated but that they could obtain necessary supplies from the same source as the military.

In conclusion, all sites of malarial segregation camps, permanent hospitals and depots, and the camps of coloured troops and Chinese labourers would have to be carefully chosen. In the event of such places being compulsorily near Anopheline waters it would be necessary to put rigorously into practice the usual preventive measures.

ACKNOWLEDGMENTS.

The writer desires to record, with grateful acknowledgments, the names of those officers, non-commissioned officers and men who so willingly assisted in the survey and, at the same time, to note the nature of the services rendered. Major Dale, D.A.D.M.S. (Sanitation) Second Army, was responsible in arranging that my movements were free and unhampered. He also collected and forwarded specimens. The following officers commanding sanitary sections of the R.A.M.C., as well as affording me pleasant hospitality, arranged means of transport and detailed assistants from their units: Captains Porter,

Bryce, Murray, Reed, Sands and Lieutenant Regan. Specimens and information were received from Captains Bain, Rankin, Johnstone, Armitage, R.A.M.C. and Lieutenants MacDonald and Saltmarsh, R.A.M.C. (T.F.) (London Sanitary Coy.). The assistant surveyors who performed so much intelligent work were Corporal Barnes, Lance-Corporals C. Roe, W. Roe and Gudger and Privates Hargreaves and Walden all of the sanitary service.

SUMMARY.

(1) The object of the investigation was to ascertain the condition of the Second Army area of Southern Flanders in regard to Anopheline mosquitoes.

(2) The data of this report have been derived from three sources (1) from the writer's surveys during the summers of 1915 and 1916; (2) from the writer's survey of September 1918; (3) from various collectors the bulk of whose specimens were collected during September 1918.

(3) Circumstances determined that the method of investigation should deal almost exclusively with breeding waters. Except on a very small scale no attempt could be made to rear larvae and pupae or capture adults.

(4) The distribution of a circular and pro-forma resulted in a certain amount of information being obtained from medical officers and officers commanding sanitary sections. Valuable assistance was also rendered by certain non-commissioned officers and men.

(5) The surveyed area of the Second Army zone bounded, roughly, by imaginary lines joining the places Millam, Woesten, Ypres, Kemmel, Armentières, Arques and Moule was about 350 square miles; most of its districts were touched upon, their areas totaling about 120 square miles; eight main districts and 12 isolated districts, representative of the country, were surveyed with varying thoroughness and skill; the main districts were the Advanced Zone, Poperinghe, Hazebrouck, Southern, Rubrouck, Central, Waterlands and Armentières.

(6) Out of 40 adult Anophelines, caught wild or reared from larvae and pupae, all proved *maculipennis*.

(7) Twenty-three *A. maculipennis*, females, were caught in one afternoon in a dark, damp medical aid-post.

(8) No seasonal preponderance of any one size of larvae was observed during September 1918.

(9) Out of about 1233 records of sites inspected, 178 refer to Anopheline haunts, 5 referring to captures of adults.

(10) Of the 173 Anopheline waters discovered 127 (73.4 %) were pools or lakes, 16 (9.25 %) were holes (shell-holes or pits), 12 (6.9 %) were running waters (9 streams, 3 canals), 8 (4.6 %) were ditches, 6 (3.5 %) were marsh, 2 (1.2 %) were receptacles (1 a tin and 1 a concrete basin); 2 records give no details.

(11) From 106 records the number of waters with few Anopheline larvae (up to about 10) was 67 (63.2 %), with numerous larvae (3 and more per dip)

22 (20.7 %); 17 (16.1 %) records are doubtful. In 2 cases the number of larvae was many thousands.

(12) On 9 occasions Anopheline larvae were found in polluted water.

(13) Anopheline larvae were found almost certainly in all waters where the visible vegetation was grass or algae or predominantly one of these. Association with water-cress was not infrequent. Ponds entirely covered with a dense mat of duckweed never gave Anopheline larvae but, at times, such pools may have open patches among marginal vegetation and in these patches the larvae have been taken. They have also been found in water which showed no visible foodstuffs.

(14) Anopheline larvae were found alone in 65 % of cases, and co-existing with Culicine larvae in 24 %, with water hog-lice (*Asellus*) in 6.5 % and with fresh-water shrimps (*Gammarus*) in 1.2 % of cases; they may also co-exist with any two or all three of these, and with fresh-water fish of many kinds.

(15) Water-boatmen (*Notonecta*) have been observed in an aquarium to prey upon and suck the juices of Anopheline larvae.

(16) All waters are suspect.

(17) It is probable that further summer work would show the number of Anopheline waters and the degree of infestation of the districts to be higher than the results here cited.

(18) Anophelines have been found generally distributed all over the area and in close proximity to billets. In 4 districts out of 20 examined, none was found, but more careful search would probably reveal them.

(19) The results obtained in the Hazebrouck district are taken as representative of the approximate degree of Anopheline infestation to which the Second Army area attained. The findings are (1) 14 % (1 in 7) of waters examined were Anopheline; (2) there were 5 Anopheline waters per square mile; (3) 25 % of Anopheline waters contained numerous larvae and 56 % contained few; of the remainder there is insufficient record.

(20) Generally speaking, and as far as information of the conditions obtaining in other regions in the temperate zone permits comparison, the degree of Anopheline infestation of the whole area appears to be low; compared with sub-tropical regions formerly war zones—Italy, Macedonia, and Palestine—it is very low. Two districts, Moule and Arques, showed waters highly infested, both being in the neighbourhood of hospitals. The ditches of the Waterlands district showed a low degree of infestation.

(21) The nature and character of the Anopheline waters render them amenable to treatment by dragging of weeds and oiling and, military exigencies permitting, sufficient preventive measures could be instituted to bring Anophelines under control.

(22) Notwithstanding that the area has been densely populated with a great variety of malarial troops and that a few cases of "primary" malaria have occurred, an epidemic of malaria among troops or civilians is unlikely.

(23) In instituting preventive work under war-conditions cognizance

should be taken of conditions in which Anopheline waters are the sole watering places for military and civilian needs; also, sites for hospitals and large permanent camps likely to house malarial subjects should be carefully chosen and kept free from Anophelines.

CONCLUSIONS.

- (1) The commonest Anopheline mosquito in the area is *A. maculipennis*.
- (2) The proportion of Anopheline waters in this area reached 14 %, i.e. 1 pool in every 7 is Anopheline.
- (3) The number of Anopheline waters per square mile reached 5.
- (4) The proportion of Anopheline waters with numerous larvae reached 25 %.
- (5) The degree of Anopheline infestation appears to be low, absolutely, and in comparison with conditions in sub-tropical countries, very low.
- (6) Two districts, Moulle and Arques, are exceptionally highly infested for this area.
- (7) All waters, particularly those with vegetation consisting of grass or algae, are suspects.
- (8) An epidemic of malaria is unlikely in this area.
- (9) Military exigencies permitting, the problem of controlling Anophelines in the area ought not to be difficult.

ON THE CLASSIFICATION OF THE ASCARIDAE.

I.—THE SYSTEMATIC VALUE OF CERTAIN CHARACTERS OF THE ALIMENTARY CANAL.

By H. A. BAYLIS, M.A.

(With 1 Text-figure.)

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INTRODUCTION.

THE great French helminthologist, Dujardin, appears to have been the first to suggest a grouping of the genus "*Ascaris*" according to the structure of the alimentary canal. As is, unhappily, only too well known to modern helminthologists, little attention was paid to internal structures by the earlier workers, whose numerous descriptions and classifications were based almost entirely upon external characters. It is now recognized that a natural system must be based on a survey of the whole structure of the animal, both external and internal, and a re-examination of many of the older species becomes extremely important.

While some families of Nematodes have received considerable attention in recent years, and materials for a natural grouping are gradually accumulating, the Ascarid family seems to have been comparatively neglected, perhaps because of the extremely large number of species, to which every year still more are being added, and which seems to make it an almost hopeless task to reduce them to a natural, orderly and convenient systematic arrangement. Hall (1916), in the course of some introductory remarks to his valuable revision of the Nematodes of Rodents, has mentioned that in the index catalogue of the Zoological Division of the United States Bureau of Animal Industry something like a thousand species are enumerated under the generic name "*Ascaris*." Stossich (1896), in his monograph of the Linnean genus, mentions 218 species. Of course, many of the so-called "species" are no doubt synonyms, misdeterminations, or otherwise inadmissible; but, even allowing for this, the "genus" is intolerably unwieldy, and it is clearly time that effective steps were taken to split it up into smaller groups, if only for the sake of convenience.

The process of splitting-up has been carried on in a desultory manner since Dujardin's day, but has not, up to the present, led to any highly satisfactory results. No uniform system has been adopted by the various workers

who have approached the subject, and the limits and contents of the families and subfamilies that have been created are very ill-defined. The present position, in general, is that the Linnean genus *Ascaris* has risen to the rank of a superfamily, Ascaroidea, with the families Ascaridae, Heterakidae and Oxyuridae. These families, again, have been variously subdivided into subfamilies, while there is a tendency, as always in the progress of systematic zoology, for the subfamilies themselves to be raised to the rank of families.

The System of Dujardin.

We may now return to the consideration of the system outlined by Dujardin (1845). Having separated off as "subgenera" the forms which now compose the families Heterakidae and Oxyuridae from the "true Ascarids," Dujardin places the latter in a "subgenus," *Ascaris*, which he further divides into four sections, taking the structure of the alimentary canal as a basis for the classification. His four sections are as follows (translating as closely as possible the original characterizations):

Section 1. "Ascarids with simple oesophagus with or without ventriculus, but without pyloric appendices."

In this section are placed the whole of the forms known from mammals, the great majority of those from birds, reptiles and fishes, and one from an insect.

Section 2. "True Ascarids in which the oesophagus is followed by a more or less distinct ventriculus and accompanied by a pyloric caecum or appendix springing from the intestine."

This section comprises the following forms:

	HOSTS
<i>A. gypina</i> Duj.	Vultures.
<i>A. depressa</i> Rud.	<i>Falco</i> , etc.
<i>A. spiralis</i> (Zeder)	Owls.
<i>A. ensicaudata</i> (Zeder)	<i>Turdus</i> spp.
<i>A. crenata</i> (Zeder) (prob. = <i>ensicaudata</i>)	<i>Sturnus</i> .
<i>A. heteroura</i> Crepl.	<i>Charadrius</i> , etc.
<i>A. semiteres</i> (Zeder)	<i>Vanellus</i> , etc.
<i>A. praelonga</i> Duj.	<i>Colymbus</i> .
<i>A. crassa</i> Deslongchamps	<i>Anas</i> , etc.
<i>A. constricta</i> Rud.	<i>Trachinus</i> .
<i>A. incurva</i> Rud.	<i>Xiphias</i> .
<i>A. ecaudata</i> Duj.	<i>Conger</i> .

Section 3. "True Ascarids in which the oesophagus is prolonged by a pyloric caecum or appendix alongside of the intestine, and itself accompanied by another caecum springing from the intestine and forwardly directed."

Here are placed the following species:

	HOSTS
<i>A. spiculigera</i> Rud.	Cormorant, Pelican.
<i>A. pedum</i> Deslongchamps	<i>Scomber</i> .
<i>A. obtusocaudata</i> (Zeder)	<i>Salmo</i> , etc.
<i>A. adunca</i> Rud.	<i>Clupea</i> .
<i>A. clavata</i> Rud.	<i>Gadus</i> , etc.

Section 4. "True Ascarids having a single pyloric caecum or appendix springing from the oesophagus, posteriorly, alongside of the intestine."

A single species,

A. acus Bloch.

Host

The Pike (*Esox*).

This résumé of Dujardin's system has been given because it seems to have been generally either forgotten or ignored by more recent workers, some of whom have actually created new genera for forms having exactly the characters given by Dujardin for one or another of his Sections 2, 3 and 4, without mentioning the fact that Dujardin had already noticed them, and without comparing the new forms with those included in Dujardin's groups.

During recent years an attempt has been made by Railliet and Henry (1912) to group together all the Ascarids in which oesophageal or intestinal diverticula occur. They thus created the subfamily Heterocheilinae, which they have subsequently (1915) shown a desire to elevate to the rank of a family, Heterocheilidae. More recently still, Geddoelst (1916) has given a dichotomous table of forms referred by him to the Heterocheilinae (apparently not accepting the group as of family rank) to which he has added a new genus, *Dujardinia*, for the reception of *Ascaris helicina* Molin. The table is based on the following features, which are here given in the supposed order of importance:

- (1) Presence or absence of intestinal and oesophageal caeca.
- (2) Presence or absence of interlabia.
- (3) Presence or absence of dentigerous ridges.

In the course of the following remarks it will be necessary to inquire whether this subfamily (or family) can be regarded as a natural group, and to see, if possible, to what extent the presence or absence of caeca connected with the alimentary canal provides a sound basis for classification.

Data derived from a re-examination of species.

With the idea of obtaining some more definite knowledge of the occurrence of these modifications of structure in the alimentary canal, and of the relationships, if any, between the forms in which they are found, a number of species of Ascarids available in the British Museum have been re-examined expressly from this point of view, note being also taken of the presence or absence of interlabia and dentigerous ridges—points usually assumed to be of systematic value. The list of species so examined at present is very limited, but the results already appear to the writer to indicate that the occurrence of such structures is more widespread among the Ascaridae than has hitherto been realised, and that it may have a very important bearing upon the ultimate systematic grouping of these forms.

The species¹ of which specimens have been re-examined at present are the following:

	HOSTS OF ACTUAL MATERIAL
<i>Ascaris aucta</i> Rud.	<i>Blennius viviparus</i> , <i>Rhombus punctatus</i> .
„ <i>decipiens</i> Krabbe	<i>Otaria jubata</i> .
„ <i>depressa</i> (Zed.)	<i>Gyps fulvus</i> , <i>Accipiter nisus</i> , "eagle."
„ <i>colura</i> Baylis	<i>Lophoaëtus occipitalis</i> .
„ <i>ensicaudata</i> (Zed.)	<i>Turdus merula</i> , <i>T. musicus</i> , <i>Sturnus vulgaris</i> .
„ <i>halichoris</i> Owen	<i>Dugong</i> , sp.
„ <i>holoptera</i> Rud.	<i>Testudo graeca</i> , <i>T. mauretanica</i> , <i>T. geometrica</i> , <i>T.</i> sp.
„ <i>microcephala</i> Rud.	<i>Ardea cinerea</i> .
„ <i>rosmari</i> Baylis (= <i>A. bicolor</i> Baird)	<i>Odobaeus rosmarus</i> .
„ <i>semiteres</i> (Zed.)	<i>Vanellus cristatus</i> .
„ <i>serpentulus</i> Rud. (? = <i>A. ardeae</i> Froel.)	<i>Ardea cinerea</i> .
„ <i>similis</i> Baird	A seal (Antarctic).
<i>Contracaecum spiculigerum</i> (Rud.)	<i>Phalacrocorax verrucosus</i> , <i>P. campbelli</i> , <i>P.</i> sp.
<i>Dujardinia helicina</i> (Molin)	<i>Crocodilus niloticus</i> .
<i>Kathleena osculata</i> (Rud.)	<i>Hydrurga leptonyx</i> .
„ <i>radiata</i> (v. Linst.)	<i>Leptonychotes weddelli</i> .
„ <i>rodhaini</i> Gedoelst	<i>Plotus rufus</i> .
„ <i>tricuspis</i> Gedoelst	<i>Phalacrocorax africana</i> .
<i>Porrocaecum crassum</i> (Deslongchamps)	<i>Anas boschas dom.</i>
<i>Terranova antarctica</i> Leiper and Atkinson	<i>Mustelus antarcticus</i> .

Among the forms just enumerated, the hosts of which include mammals, birds, reptiles and fishes, no example of the typical, simple, Ascarid structure of the alimentary canal was met with. In every case some modification of the oesophagus was present, or some caecum or appendage either of the oesophagus or of the intestine, or both.

Five main types of structure were encountered:

I. Oesophagus muscular throughout, opening directly into the intestine, without posterior ventriculus or distinct bulb. A forwardly-directed caecum springs from the intestine. No oesophageal appendix. Examples: *Ascaris holoptera*, *A. colura*.

II. Oesophagus slender, with a more or less distinct globular bulb at the base. The intestine is produced forwards as a long caecum. No oesophageal appendix. Examples: *Ascaris halichoris*, *Dujardinia helicina*.

III. Oesophagus with a posterior glandular portion, or ventriculus, of elongate or oblong shape and often bent in a sigmoid manner. No oesophageal or intestinal caeca. Examples: *Ascaris rosmari*, *A. similis*.

¹ The determination cannot in all cases be vouched for with certainty, though no undue suspicion attaches to any of the specimens. Those of *Ascaris colura*, *A. rosmari*, *A. similis* and *Terranova antarctica* are the type-specimens of the species in question. Those of *Kathleena osculata* and *K. radiata* were determined by Leiper and Atkinson, being part of the "Terra Nova" collection. The examples of *A. decipiens* had been misdetermined by von Linstow as "*A. simplex* Rud.," and the present determination is the writer's. Two out of the three sets of *C. spiculigerum* (*Ascaris spiculigera* Rud.), belonging to the "Challenger" and "Discovery" collections respectively, were determined by von Linstow. Named specimens of *Porrocaecum crassum* were kindly supplied by Prof. Railliet.

IV. Oesophagus with a posterior glandular portion, or ventriculus, often bent so as to open into the intestine laterally. An intestinal caecum present. No oesophageal appendix. Examples: *Ascaris decipiens*, *A. depressa*, *A. ensicaudata*, *Porrocaecum crassum*, *Terranova antarctica*, probably *Ascaris semiteres*, *A. serpentulus*¹.

V. Oesophagus with a reduced posterior ventriculus, giving off a backwardly-directed glandular appendix. An intestinal caecum also present. Examples: *Ascaris aucta*, *A. microcephala*, *Contracaecum spiculigerum*, *Kathleena osculata*, *K. radiata*, *K. rodhaini*, *K. tricuspis*.

The subfamily Heterocheilinae, Railliet and Henry.

We may next consider the classification outlined by Railliet and Henry (1912), to which reference has already been made. These authors divided the family Ascaridae into the following subfamilies:

(1) ASCARINAE, to include *Ascaris* L. and other genera with a simple alimentary canal.

(2) ANISAKINAE, to include *Anisakis* Duj., 1845 (= *Peritrachelius* Dies., 1851; = *Conocephalus* Dies., 1861) and perhaps *Crossocephalus* Railliet, 1909 (= *Pterocephalus* v. Linst., 1899).

(3) HETEROCHEILINAE, to include provisionally all the forms with oesophageal or intestinal caeca. *Heterocheilus* Dies., 1839; *Typhlophorus* v. Linst., 1906; *Porrocaecum* Railliet and Henry, 1912; *Crossophorus* Hempr. and Ehbr., 1828; *Lecanocephalus* Dies., 1839; *Contracaecum* Railliet and Henry, 1912. To these were subsequently added (1915) the genera *Terranova* and *Kathleena* of Leiper and Atkinson, 1914, and *Raphidascaris* Railliet and Henry, 1915.

With the first subfamily it is not intended to deal at present. Of the forms included in the last two subfamilies, it is important to note the following features.

Anisakis (type-species (?) "*Ascaris simplex* Rud." of Dujardin—type-specimens renamed *A. dussumieri* by van Beneden, 1870—? synonym, *Peritrachelius* [*Conocephalus*] *typicus* (Dies., 1860); Host, *Delphinus*). The main character upon which Dujardin's subgenus *Anisakis* was based was the presence of two spicules of unequal length in the male. The descriptions of *A. dussumieri* and of *Ascaris* [*Peritrachelius*] *typica* show, however, that the oesophagus possesses a posterior ventriculus of different histological appearance from the anterior, muscular portion, and that oesophageal and intestinal caeca are absent. (See Stiles and Hassall (1899), where the anatomy and the complicated synonymy are fully dealt with.)

Crossocephalus, as more recent researches (Gedoelst (1916); Baylis (1919, a))

¹ The material available for the study of the last two forms did not permit of satisfactory examination.

have shown, has no close relationship with any of the Ascaridae, and probably belongs to the Oxyuridae.

Heterocheilus (type-species, *H. tunicatus* Dies., 1839, from *Manatus exunguis*) has a very peculiar cuticular swelling behind the lips, consisting of a series of longitudinal ribs; while the lips themselves, from Diesing's figures, seem to be of an unusual type. The oesophagus has a bulb at the base, and there is an intestinal caecum running forwards.

Typhlophorus (type-species, *T. lamellaris* v. Linst., 1906, from *Gavialis gangeticus*). From the brief description of this form, and from the figures, it appears to be closely related to *Heterocheilus*. There is a very similar longitudinally-ribbed cuticular swelling behind the lips. A long intestinal caecum is present, but the structure of the oesophagus is not described.

Porrocaecum (type-species, *P. [Ascaris] crassum* (Deslongchamps), from ducks). In the structure of the alimentary canal this genus corresponds to Dujardin's Section 2, which includes the type-species, and also to what has just now been described as "type IV."

Crossophorus is a genus of so highly specialised a type that it seems to have little or nothing in common with the other genera. It certainly possesses two long intestinal caeca, but in several features (see Baylis (1919, b)) it appears to be quite isolated.

Lecanocephalus. The correct generic name, as Railliet and Henry (1915) have pointed out, is *Goezia* Zeder, 1800 (type-species, *G. ascaroides* (Goeze, 1782), from *Silurus glanis*). This genus is characterised by having the cuticle armed with numerous transverse rings of spines, and by the peculiar, flattened shape of the lips, which are unlike those of any other known Ascarid. For these reasons it may be doubted whether it bears any close relationship to most of the genera among which it has been placed. Its claim to this position is based on its possession of a long glandular oesophageal appendix and a short intestinal caecum, in which respects it resembles *Contracaecum*.

Contracaecum (type-species, *C. [Ascaris] spiculigerum* (Rud., 1809), from the cormorant, etc.) corresponds, in the structure of the alimentary canal, to Dujardin's Section 3, and to "type V" in the list given above.

Terranova (type-species, *T. antarctica* Leiper and Atkinson, 1914, from a shark, *Mustelus antarcticus*). The characters upon which this genus was based were "three large simple lips. No interlabia. Oesophagus simple. Gut with anterior caecal prolongation. No oesophageal appendage." A re-examination of the type-specimen, however, shows that the statement that the oesophagus is simple was erroneous, and that, on the contrary, it has a specialised ventricular portion, 1.4 mm. long, and therefore the structure is of the same type as in *Porrocaecum*.

Kathleena (type-species, *K. osculata* (Rud., 1802), from seals). On comparing examples of the two type-species, the characters of this genus appear to be identical with those of *Contracaecum*.

Raphidascaris (type-species, *R. acus* (Bloch, 1779), from the pike) is

characterised by the possession of an oesophageal appendix, but no intestinal caecum.

From this survey it appears doubtful whether the subfamily Heterocheilinae, as it stands, can be regarded as a satisfactory or natural group. We have seen that *Crossophorus* probably ought to be removed to a position by itself. The genera *Heterocheilus*, *Typhlophorus* and *Goezia* (= *Lecanocephalus*) also seem to be clearly marked off from the rest of the forms by peculiarities of external features—all the remainder being of typical "Ascarid" appearance externally.

There can be little question that *Terranova* is generically identical with *Porrocaecum*, and *Kathleena* with *Contracaecum*. Hence, according to the law of priority, the names *Terranova* and *Kathleena* fall into synonymy.

Setting aside all the aberrant forms mentioned above, the only genera among the original Heterocheilinae with which we are immediately concerned are *Porrocaecum*, *Contracaecum* and *Raphidascaris*. These genera, together with *Anisakis*, and with the addition of certain other forms to be mentioned, seem to compose a more natural group, united by possessing the general outward appearance of an "Ascaris," but with certain modifications of the alimentary canal which mark them off from the Ascarinae.

Proposed new arrangement.

The new group which it is proposed to set up comprises the type-genus of the former subfamily Anisakinae and part of the former subfamily Heterocheilinae. The forms which are to be included in it may be divided into two sections, according to the presence or absence of a specialised "ventriculus," forming a posterior division of the oesophagus. This structure, for which Dujardin's term may conveniently be used, has been neglected since his time as a feature of systematic value. It seems to the writer, however, that it deserves the consideration which Dujardin was disposed to give to it, and that it is probably of greater phylogenetic importance than the presence or absence of caecal appendages. Although no special attention has as yet been devoted to the ventriculus of Ascarids from the histological or physiological standpoints, it is interesting to observe that in a nematode of another family, *Camallanus*, in which a very similar organ exists, a recent writer (Magath (1919)) has suggested that it is concerned in the excretory processes. In the species dealt with it seems to have some structural connection with the excretory apparatus. Now in some of the Ascarids in which a ventriculus occurs we find it associated with a peculiar type of excretory apparatus, terminating in a long, unpaired, unicellular "gland," opening close to the lips. This is the case, at all events, with a number of the forms included here under the names *Anisakis*, *Porrocaecum* and *Contracaecum*, and will very possibly be found to be characteristic of all the forms in which there is a ventriculus. Pending fuller investigation, therefore, it is suggested that all

the forms with a true ventriculus should be placed together. It may prove that the forms without a true ventriculus ought to be entirely separated from those possessing it, and that their inclusion in the same subfamily is too artificial. Certain forms of this type may, however, be placed here at present provisionally. The true relationships of all the genera and species can, of course, only be estimated after a much fuller inquiry into their entire anatomy.

The following arrangement is suggested:

ANISAKINAE Railliet and Henry, 1912, *emend.* Baylis, 1920.

Ascaridae having the general external appearance of an *Ascaris*, *i.e.* with a smooth cuticle, transversely striated but without cuticular spines or other raised structures. The oesophagus may or may not be divided into an anterior muscular portion and a posterior ventriculus of different histological structure. When the latter is absent (and frequently when it is present) there is an anterior caecum, springing from the intestine and lying alongside of the oesophagus. A posterior caecum or solid glandular appendix may also be developed in connection with the ventricular portion of the oesophagus. Interlabia may be present or absent, and when present show various degrees of development. Dentigerous ridges on the lips may also be present or absent. The species are parasitic in the alimentary canal of mammals, birds, reptiles and fishes, and the majority are found in aquatic or at least fish-eating hosts. There is reason to believe that in some cases, and perhaps in all, an intermediate host is required for their development, and that, in the case of species inhabiting fish-eating animals when adult, the intermediate host is a fish.

Type-genus—*Anisakis* Duj., 1845.

The following genera may be enumerated:

(1) *Anisakis* Duj., 1845 [= *Peritrachelius* Dies., 1851; = *Conocephalus* Dies., 1861].

Oesophagus with anterior muscular portion and posterior ventriculus, the latter being oblong or sigmoid in shape. No oesophageal or intestinal caecum. Interlabia absent. Dentigerous ridges present. Spicules of male sometimes unequal.

Hab.—stomach and intestine of marine mammals.

With the following species:

A. dussumieri van Ben., 1870 (= *A. simplex* Duj., 1845, *nec* Rudolphi, 1809) (genotype) from a dolphin.

A. typica (Dies., 1860) from *Delphinus*, *Phocaena*, *Prodelphinus* (perhaps identical with the preceding).

A. insignis (Dies., 1851) from *Inia geoffroyi* (= *Delphinus amazonicus*).

A. similis (Baird, 1853) from a seal (Antarctic).

A. rosmari (Baylis, 1916) [= *Ascaris bicolor* Baird, 1868] from the Walrus (*Odobenus rosmarus*).

A. simplex (Rud. 1809) from *Balaenoptera*, *Delphinus*, etc.

? *A. kükenhalii* (Cobb, 1888) from *Delphinapterus* (sp. inq.; perhaps = *A. simplex*).

(2) *Raphidascaris* Railliet and Henry, 1915¹.

Oesophagus with anterior muscular portion and small posterior ventriculus. From the latter springs a small posterior appendix. No intestinal caecum. Interlabia present. Dentigerous ridges absent.

Hab.—intestine and stomach of fishes.

Species:

R. acus (Bloch, 1779) (genotype) from the pike (*Esox*).

(3) *Porrocaecum* Railliet and Henry, 1912. (Syn. *Terranova* Leiper and Atkinson, 1914.)

Oesophagus with anterior muscular portion and posterior ventriculus of oblong shape, the latter short in the genotype, but in other species frequently long and bent at an angle so as to open into the intestine laterally. An intestinal caecum present. No oesophageal appendix. Interlabia present, usually small. Dentigerous ridges present.

Hab.—intestine of birds, marine mammals and fishes.

Species:

P. crassum (Deslongchamps, 1824) (genotype) from ducks.

P. depressum (Zed., 1800) from birds of prey (*Falco*, etc.).

P. ensicaudatum (Zed., 1800)² from *Turdus*, *Sturnus*.

P. decipiens (Krabbe, 1878) from seals.

P. antarcticum (Leiper and Atkinson, 1914) from *Mustelus antarcticus*, and probably also

P. semiteres (Zed., 1800)³ from *Vanellus*, etc.

P. serpentulus (Rud., 1809) from *Ardea* sp.

P. heteroura (Crepl., 1829) from *Charadrius*, etc.

P. spirale (Zed., 1803) from owls.

P. praelongum (Duj., 1845) from *Colymbus*.

Certain species from fishes, with this type of alimentary canal, mentioned by Dujardin, require further investigation.

(4) *Contracaecum* Railliet and Henry, 1912. (Syn. *Kathleena* Leiper and Atkinson, 1914.)

Oesophagus with reduced posterior ventriculus, giving off a solid posterior appendix. An intestinal caecum present. Interlabia present, usually very well-developed. Dentigerous ridges absent⁴.

¹ Ward and Magath (1916) have described a new genus, *Hysterothylacium* (type-species *H. brachyurum* Ward and Magath, from the "black bass"), which is stated to belong to the Heterocheilidae and to have the following characters: "Esophagus long, slender, with terminal spherical bulb. Intestine with short simple cecum, arising from anterior end of intestine, directed posteriad." The oesophageal bulb is said to contain three teeth. The authors do not make any comparison between this form and *Raphidascaris*, but it appears not unlikely that the two forms are related. It is important to note, however, that the caecum is said to belong to the intestine and not to the oesophagus. Wigdor (1918) has described a second species, *H. cayugensis*, from *Esox* and *Ameiurus*, which, from its description, can hardly be distinguished from *Raphidascaris acus*.

² In *P. ensicaudatum* the caecum is very small and almost rudimentary, at least in the examples seen.

³ The single specimen of *P. semiteres* available for study proved too opaque for the structure to be made out with certainty.

⁴ Geddoelst's (1916) statement that dentigerous ridges are present in *Contracaecum* is ap-

Hab.—intestine of fish-eating mammals and birds, and of fishes.

Species:

- C. spiculigerum* (Rud., 1809) (genotype) from cormorants, etc.
- C. osculatum* (Rud., 1802) from seals.
- C. radiatum* (v. Linst., 1906) from seals.
- C. rectangulum* (v. Linst., 1906) from seals.
- C. scotti* (Leiper and Atkinson, 1914) from *Diomedea*.
- C. microcephalum* (Rud., 1809) from *Ardea*, etc.
- C. rodhaini* (Gedoelst, 1916) from *Plotus rufus*.
- C. auctum* (Rud., 1809)¹ from *Blennius*, etc.
- C. clavatum* (Rud., 1819) from various fishes.
- C. aduncum* (Rud., 1809) from *Clupea*.

And probably other species from fishes (see Dujardin (1845), pp. 208–211).

Gedoelst (1916) has compiled a list of species to be referred to *Kathleena*. These should probably all be included here, and we have therefore the following additional species:

- C. lobulatum* (Schneider, 1866) from *Platanista gangetica*.
- C. falcigerum* (Railliet and Henry, 1907) from seals.
- C. multipapillatum* (v. Drasche, 1882) from *Tantalus loculator*.
- C. micropapillatum* (Stossich, 1890) from *Pelecanus* spp.
- C. ovale* (v. Linst., 1907) from *Podiceps cristatus*.
- C. rosarium* (Connal, 1912) from *Nycticorax* sp.
- C. tricuspe* (Gedoelst, 1916) from *Ardea* sp.
- C. punctatum* (Gedoelst, 1916) from ?*Pseudotantalus ibis*.

Kathleena arcuata Gedoelst, 1916, from *Ardea*, sp., appears to the writer to be identical with *C. microcephalum* (Rud.).

(5) *Dujardinia* Gedoelst, 1916.

Oesophagus with a small posterior spherical bulb. An intestinal caecum present. No oesophageal appendix. Interlabia present. Dentigerous ridges absent.

Species:

- D. helicina* (Molin, 1860) (genotype) from *Crocodilus* spp.
- D. halichoris* (Owen, 1833) from *Dugong* spp.

(6) *Angusticaecum*, n.g.

Oesophagus without ventriculus or distinct bulb. A long slender caecum springs from the intestine a little behind its origin. No oesophageal appendix. Interlabia absent. Dentigerous ridges present.

Species:

- A. holopterum* (Rud., 1819) (genotype) from *Testudo* spp.
- A. [Porrocaecum] numidicum* (Seurat, 1917) from *Rana ridibunda*.

(7) *Amplicaecum*, n.g.

Oesophagus without ventriculus or distinct bulb. A wide intestinal caecum

parently an error. They have not been observed in the species examined, and the distinction between *Contracaecum* and *Kathleena*, based on this point, does not appear to hold good.

¹ *C. auctum* has the interlabia much reduced.

present. No oesophageal appendix. Small interlabia present. Dentigerous ridges present.

Species:

A. colurum (Baylis, 1919) (genotype) from *Lophoaëtus occipitalis*.

A table may now be given which will serve to distinguish the genera mentioned, and also show to some extent the relationships supposed to exist between them.

A. Oesophagus divided into an anterior muscular portion and a posterior ventriculus, the latter being oblong or sigmoid in shape, or having a posterior appendix.

(a) An intestinal caecum present.

(α) An oesophageal appendix present...*Contracaecum*.

(β) Oesophageal appendix absent...*Porrocaecum*.

(b) No intestinal caecum.

(α) An oesophageal appendix present...*Raphidascaris*.

(β) Oesophageal appendix absent...*Anisakis*.

B. Oesophagus without ventriculus, with or without a small but distinct posterior bulb. The latter, if present, spherical and without a posterior appendix. An intestinal caecum present.

(a) A distinct spherical oesophageal bulb present...*Dujardinia*.

(b) Distinct oesophageal bulb absent.

(α) Small interlabia present...*Amplicaecum*.

(β) Interlabia absent...*Angusticaecum*.

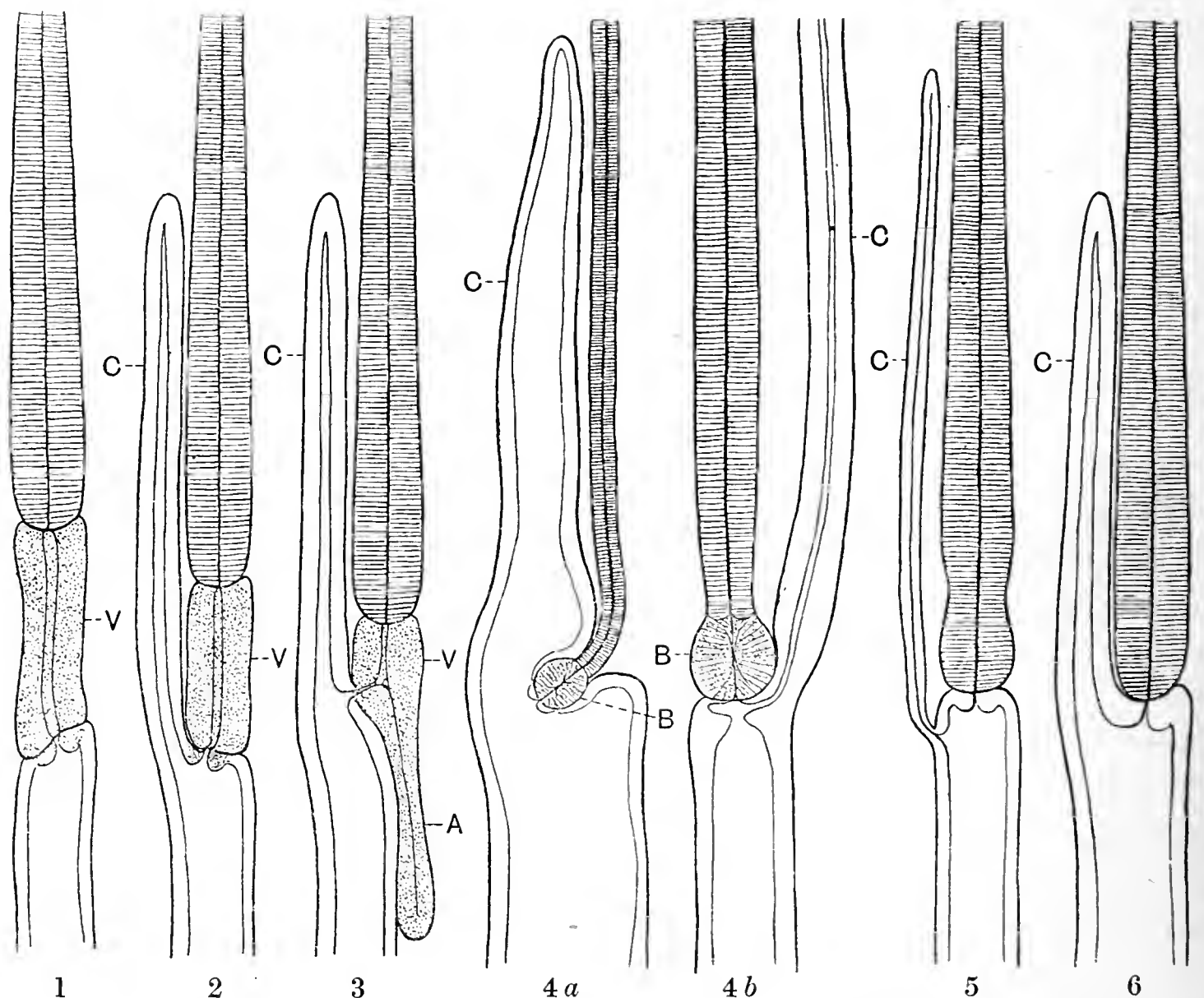
We have now to consider the position of the genera which were removed from the rest of the "Heterocheilinae"—*Heterocheilus*, *Typhlophorus*, *Goezia* and *Crossophorus*. The last-named genus seems so unlike any other Ascarid at present known that it may at once be placed in a subfamily by itself, which may be called CROSSOPHORINAE, n. subfam.

As regards the remaining three genera, it may be well to recall that von Drasche (1884) placed *Lecanocephalus* (= *Goezia*) and *Heterocheilus* in separate categories, which he named Lecanocephalidea and Heterocheilidea respectively. Considering the peculiarities of the genera in question, it seems not unreasonable to retain von Drasche's arrangement, and altering his names in accordance with the plan of modern nomenclature, to place *Heterocheilus* and *Typhlophorus* together in a subfamily HETEROCHEILINAE (or, in other words, to restrict Railliet and Henry's (1912) subfamily to these two genera), and *Goezia* by itself in another subfamily, GOEZIINAE.

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A series of diagrams illustrating certain types of structure met with in the alimentary canal of Ascaridae. The shaded portion represents the hinder part of the oesophagus.

A, oesophageal appendix; B, oesophageal bulb; C, intestinal caecum; V, ventriculus.

1, *Anisakis*; 2, *Porrocaecum*; 3, *Contracaecum*; 4, *Dujardinia* (4a, *D. helicina*; 4b, *D. hali-choris*); 5, *Angusticaecum*; 6, *Amplicaecum*.

No specimens of *Raphidasca* having been available, the structure found in this genus has not been figured. It is presumably like (3), but without the intestinal caecum.

SARCOPTIC SCABIES IN MAN AND ANIMALS.

A CRITICAL SURVEY OF OUR PRESENT KNOWLEDGE REGARDING
THE ACARI CONCERNED¹.

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(From the Quick Laboratory, Cambridge.)

(With Plate XV and 10 Text-figures.)

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INTRODUCTION.

SARCOPTIC SCABIES in man and in domestic and wild mammals has engaged the attention of a large number of investigators since its true nature was finally established about the year 1835. The subject has been approached from the medical, the veterinary and the entomological side, and the literature concerning it has attained very large dimensions. Nevertheless it cannot be said that our knowledge of the matter is in a satisfactory condition, since no two writers agree entirely in their accounts of the morphology and bionomics of the human itch-mite, and the most diverse views are held as to the inter-relation of the forms which infest man and the lower animals.

Munro (1919) goes so far as to say that “no satisfactory description of any of the members of the genus *Sarcoptes* has yet been given.” If this be so it is not for want of patient investigation by a number of scientific men exceedingly well equipped for the task, but the creatures concerned are so small and so slightly chitinised, and the minute differences between them are

¹ Work done with the aid of a grant from the Ministry of Agriculture and Fisheries.

so difficult of observation that their thorough investigation is a matter of great intrinsic difficulty.

A revision of the subject is urgently needed, with the examination of fresh material, and a full knowledge of the results obtained hitherto. Such a revision will be greatly facilitated if the conclusions of previous workers are collated and presented in a compact form, and their chief discrepancies clearly indicated, and this is the immediate object of the present article.

HISTORICAL.

Real knowledge concerning the nature and cause of scabies dates from the 16th century. Numerous passages in the writings of the ancients seem to prove an acquaintance with the disease, which has no doubt afflicted man and domestic animals from time immemorial, but until the various skin diseases were discriminated and the parasites to which some of them are attributable had been discovered and studied, such allusions were bound to be vague and unsatisfactory.

Scaliger (1557) is the first writer to show an exact knowledge of the subject. He describes how the parasite "lodges under the epidermis and burns by the galleries it burrows." Ambroise Paré (1564) explains how the mites can be extracted by the aid of a needle. Aldrovandus (1596) gives an unmistakeable account of the disease, so that it is clear that much accurate information on the matter existed by the end of the 16th century.

Further progress was made during the 17th century. The Englishman Thomas Moffett (or Muffet, Moufet, Moufeti^{us}) (1634) states that the mites do not inhabit the pustules but are to be found near-by—an observation which would have saved much trouble if subsequent investigators had borne it in mind—and Hauptmann (1657), in a work on the Waters of Wolkenstein, gave for the first time a (very inaccurate) figure of the parasite.

Then Bonomo¹ (perhaps a pseudonym for Cestoni), in a letter to Redi written in 1687, treated the subject in a manner remarkable for its admixture of truth and error, and his figure held the field till the middle of the next century as the classical representation of the sarcopt, being given a new lease of life by its use by Mead in *Philosophical Transactions* for 1702. So the matter rested till the advent of Linnaeus, who gave a good account of the mite and its habits, though he vacillated with regard to its name and systematic position, calling it *Acarus humanus subcutaneous* (1734) and *Acarus scabiei* with a doubtfully distinct form *Acarus exulcerans* parasitic on animals (1758), but to the end confusing it with the flour-mite *Tyroglyphus*.

The first thorough-going study of the mite as the exclusive cause of scabies is that of De Geer (1778), and in 1786 the Hanoverian physician

¹ The letter to Redi was signed Giovan. Cosimo Bonomo. Delafond and Bourguignon (p. 89) say roundly that Cestoni wrote to Redi under this pseudonym, and their contemporaries were of the same opinion. There was, however, a Dr Cosimo Bonomo who was a pupil of and a collaborator with Cestoni. The matter is fully discussed in Fürstenberg, p. 13 *et seq.*

Wichmann¹ published his *Aetiologie der Krätze*, giving an excellent account of scabies and its cause, supported by several new observations. Then sundry writers (Pinel on the medical side and Latreille on the entomological side) took up a discussion on the systematic position of the mite, trusting to the figures of Cestoni and De Geer. Latreille founded the genus *Sarcoptes* in 1806.

The history of the subject during the early years of the 19th century is most curious. Various medical writers (Alibert, 1833; Biett, 1836; and others), overlooking Moffett's observation and examining only the vesicles and pustules, declared that they were quite unable to find the mite in numerous undoubted cases of scabies. Galès, indeed, described and figured (1812) a mite which he said inhabited the pustules, but others could not find it, and by many this re-discovery was regarded as an imposture, and in 1821 Mouronval published a volume to prove that it did not exist!

Meanwhile Walz (1809) had been investigating sheep scabies, and de St Didier (1813) and Gohier (1816) had studied horse scabies, but the doctors remained ignorant of the work done by the veterinarians.

Then Raspail attempted a revision of the whole matter as regards human scabies, and his statement that the mite found by Galès was the cheese mite, aroused great interest, and all concerned became eager to have the matter settled once for all. At this time Francis Renucci, a Corsican, accustomed from his infancy to the "chasse aux sarcoptes," was studying medicine in Paris, and had no difficulty at all in proving the existence of the mite. This re-discovery was communicated to Raspail who published (1834) a *Mémoire comparatif sur l'histoire de l'insecte de la Gale*, which, however, contained many errors. In the same year Albin Gras published a much better account of the *Sarcoptes*, and in 1835 Renucci himself presented his thesis for the doctorate with figures of the *Sarcoptes* of man, the horse, the sheep and the cat, and also a figure of the cheese mite.

This was the condition of things when in 1843 Bourguignon, who was at the Veterinary College at Alfort under Prof. Delafond, undertook his admirable study of human scabies. He handed in his *Traité entomologique* in 1846, but it was not published till 1852. Meanwhile Hebra was at work in Vienna, and Eichstedt in Germany. Bourguignon does not seem to have known of Eichstedt's work, which included a remarkably fine study of the galleries of *Sarcoptes*, the arrangement of the eggs in them, the phenomena of moulting etc., but he had some acquaintance with Hebra's investigations, and questions of priority arose in 1845 between Hebra and Bourguignon. Of this period also is the work by Gurlt and Hertwig on human scabies (1844).

The nature and cause of scabies was now firmly established and generally admitted, but there was much to be discovered, and a large number of students were attracted to a field in which so much interest had been aroused. Scabies

¹ According to Raspail, Wichmann's treatise is a little 12mo. volume sometimes to be found bound up with a treatise by Guldner on the Prague workhouse. This is the second edition, published 1791.

became quite fashionable as a subject for theses for the doctorate. Gerlach published his *Krätze und Räude* in 1857; Fürstenberg his *Krätzmilben der Menschen und Thiere* in 1861; and Delafond and Bourguignon their *Traité pratique* in 1862.

The fine works of Bourguignon, Gerlach and Fürstenberg are generally regarded as the classical publications on this subject, and deserve a special notice. They present a remarkable variety of style and outlook.

Bourguignon is a leisurely, elegant, philosophical—and it must be confessed sometimes prolix—writer, who works away patiently and indefatigably, without too much reference to what his contemporaries are doing, though he is learned on the ancient history of the subject. He anticipates incredulity for some of his results, but is prepared to stand by them. “Tous les faits que nous avançons ont été vus cent fois avant d’être définitivement admis, et comme beaucoup d’entre eux échapperont nécessairement au premier abord aux entomologistes, qui seraient tentés de les vérifier, car la grande habitude d’étudier le même objet vous donne à la longue une habileté, qu’on n’aurait pas dans le principe serait-on le plus habile des observateurs, nous prions ceux, qui contesteraient la présence de tel ou tel organe, de nous permettre de leur fournir la preuve de son existence” (*Traité entomologique*, p. 15). One cannot “say fairer” than that!

He pays much attention to the dorsal armature, and to the skeletal structure of the legs and rostrum. He found and described the male, and observed the tocostome of the female, and altogether did remarkable pioneer work, though his details have in many respects been proved to be inaccurate by subsequent observers with more adequate instruments.

His joint work with Delafond—the *Traité pratique*, published in 1862—is a comprehensive account of scabies—whether sarcoptic, psoroptic or chorioptic—on man and other animals as known at that date. It is divided (like Gaul) into three parts. Part I deals with classification, anatomy and development, and physiology. Part II deals with scabies in man, lemur, bear, hyaena, fox, dog, lion, cat, wombat, llama, sheep, ox and fowl. Its main theme is that carnivorous and omnivorous animals (bear, dog, hyaena, lion, cat, pig) are subject to a form of scabies transmissible to man, due to a *Sarcoptes* which is probably identical with man’s; and that herbivorous animals (horse, camel, llama, sheep) may have a *Sarcoptes* and transmit it to man, but that they are more subject to psoroptic and chorioptic scabies not so transmissible. Part III is medical, and deals with treatment.

Bourguignon made many experiments in transferring *Sarcoptes* from one animal to another, and in several passages he calls attention to what he believes is the peculiar position held by man with regard to these mites. Thus on p. 613 of the *Traité pratique* he says: “Man has the sad privilege of being free from the law which regulates all contagion of animals among themselves. His skin always offers an asylum suitable for *Sarcoptes*; his dermal fluids are always to their taste, and there is to-day no doubt that the

true cause of man's persistent scabies exists, not in contagion between diseased and healthy man, but in the frequent transmission of this disease to man by animals."

Gerlach's treatise is important (for our purpose) chiefly for its study of the horse *Sarcoptes* and its comparison with that of man, and for his description and figures of the *Sarcoptes* of the dog and the pig. He gives a figure of the burrow or gallery of the human itch-mite (very adversely criticised by Delafond and Bourguignon) and in some respects his illustrations are among the best, if minute detail be not looked for.

Out of 230 folio pages of Fürstenberg's remarkable work no fewer than 170 are occupied by a truly wonderful digest of the writings of every author, from Aristotle downwards, who had hitherto dealt with the subject. The portion of the treatise which especially concerns us is not extensive, but it is extremely important because of the minute detail with which the skeletal tissues and the armature of scales and spines are dealt with, the figures being drawn by aid of a "camera clara" of his own construction. Some of his drawings of dorsal scales and cones are magnified 700 times. Nevertheless in many of his figures he makes the extraordinary mistake of giving his *Sarcoptes* two pairs of chelicerae!

Of subsequent writers two of the most important are Robin who in 1869 published his *Mémoires sur diverses espèces d'Acarions de la Famille des Sarcoptidés*, and Mégnin, who, among numerous brochures on the Acarina, contributed several which dealt especially with this group.

All the writers of compendia on parasitology of a later date (L. G. Neumann, Blanchard, Railliet, Brumpt, Neveu-Lemaire, etc.) have necessarily dealt with the subject as far as the scope of their work permitted, and some of them, notably Railliet, have made original contributions to our knowledge. From a purely medical point of view the literature has become immense, but with this aspect of the matter we are not here especially concerned.

CLASSIFICATION AND MORPHOLOGY OF THE SARCOPTINAE.

There is as yet no generally accepted classification of the Acarina. Very much work has been done of recent years in certain groups, and a few have been monographed. There is, indeed, substantial agreement as to the general affinities of the various members of the order, but the widest differences exist as to the rank to be attributed to its subdivisions. Thus while Canestrini (*Atti Ist. Veneto*, II. 1891) admitted 34 families, Trouessart (*Rev. Sci. Nat. Ouest*, II. 1892) allowed only ten.

To come at once to the group with which we are especially concerned, the term Sarcoptidae has quite a different scope as employed by different systematists. Canestrini and Kramer (Schultze's *Tierreich*, 1899) use it in the widest sense, making it equivalent to what Banks (1904) and others regard as the super family Sarcoptoidea, while their Sarcoptinae are the whole family of Sarcoptidae as understood by the latter school, and include the following

genera: *Sarcoptes*, *Notoedres*, *Prosopodectes*, *Cnemidocoptes*, *Psoroptes*, *Psoralgæ*, *Chorioptes*, *Caparinia*, *Otodectes*. This view is far from satisfactory as it takes no account of the specially close affinity between the four first-named genera, which agree in possessing mouth-parts adapted for burrowing, and are, in fact, the forms which induce "sarcoptic scabies" in various animals. It is much more convenient, and, indeed, is the general practice, to consider the nine genera above named as constituting the SARCOPTIDÆ, of which a subfamily SARCOPTINÆ includes the four genera *Sarcoptes*, *Notoedres*, *Prosopodectes* and *Cnemidocoptes*, and it is with the Sarcoptinae in this restricted sense that we are concerned. *Prosopodectes* is a parasite only found in the ears of bats, and it is not proposed to discuss it, so that only three genera remain. Indeed since *Cnemidocoptes* is parasitic only on birds, our chief interest is in the genera *Sarcoptes* and *Notoedres*.

Sarcoptes, *Notoedres* and *Cnemidocoptes* are precisely the *Sarcoptes communes*, *Sarcoptes notoedres* and *Sarcoptes anacanthæ* of Delafond and Bourguignon (1862, p. 13).

SARCOPTES Latreille, 1806.

The *Tierreich* definition is: ♂ without anal cylinders (Analnöpfe), ♀ without copulation tubes; long unjointed ambulacra on the two anterior legs of the ♀ and the two anterior and the second posterior legs of the ♂. Anus terminal. Oviparous parasites of mammalia.

Berlese (*Acarotheca italica*, Textus, 1913) thus defines the genus:

Maris disculi copulationis foeminaeque tuberculi nulli. Ambulacra longe pedunculata, pedunculo haud articulado, in foemina sunt in pedibus primi secundique paris, in mari in primo secundo quartoque pari. Anus in margine postico abdominis apertus. Ovipari, mammalicoli, scabiem sarcopticam inducentes. Species typica *Acarus scabiei* De Geer. Totus mundus.

He admits 15 species, namely: *scabiei* De G., *canis* Gerl., *caprae* Fürst., *dromedarii* Gerv., *equi* Gerl., *ovis* Mégn., *parvulus* Can., *cuniculi* Neum., *scabiei-crustosae* Fürst., *suis* Gerl., *vulpis* Fürst., *aucheniae* Raill., *hydrochaeri* Mégn., *rupicaprae* Hering, *wombati* Raill.

Canestrini and Kramer (in Schultze's *Tierreich*, 1899) include all these together with three others, *leonis* Can., *lupi* Mégn., *furonis* Raill., in their list of species, but they consider *aucheniae*, *hydrochaeri*, *rupicaprae* and *wombati* "doubtful."

And now it will be as well to interpose at the outset the gist of a passage from Mégnin (1875, pp. 1058–1060) so significant that it is bound to affect profoundly the views of anyone engaged on a revision of the matter. He had been studying the *Sarcoptes* of the horse during and after the Franco-Prussian War (1871 and 1872), and had created a new species, *S. uncinatus*, based on:

(1) the presence of a strong claw on the inferior face of the second article of each anterior leg;

(2) the presence in the middle of the notothorax in both sexes, but

stronger in the ♂, of a quadrangular, chitinous, granular, yellowish "plastron," presenting in the middle of its anterior border two rudiments of stigmata;

(3) the presence, on the notogaster of the ♂, of two chitinous, granular, yellowish, circular, symmetrical plates between the four rows of spinules.

Continuing his studies on *Sarcoptes* collected by himself from the giraffe, gazelle, wolf etc., and on specimens furnished him by Gervais from the llama, moufflon, cabiai etc., he found in them precisely the same details of structure. Finally, on examining specimens taken from patients at the hospital of St Louis he was again able to recognise the characteristics he had thought peculiar to his new species on the horse, but so indistinct and colourless that they were not noticeable until especially looked for. His conclusion is that the "German authors" have depended on habitat and insignificant characters in founding a large number of species, while there is in reality only one species—*S. scabiei*, with a certain number of varieties. He finally remarks: "The different varieties are characterised by a difference of activity in their poisonous buccal liquid. I have just recently placed on the horse the *Sarcoptes* taken from the wolf, and they gave rise to colonies which *in ten days* invaded the whole surface of the pachyderm, giving rise to a scabies much more severe than that caused by its own proper *Sarcoptes*!"

This passage, be it remarked, long antedated the works cited above in which authors of such undoubted authority as Kramer, Canestrini and Berlese give extensive lists of distinct species of *Sarcoptes*.

The plan which seems best adapted to our purpose is to give in the first place a full account of the *Sarcoptes* of man as far as it is known, indicating differences of opinion where they exist, and then to deal briefly with the forms which affect other animals. Many of the latter are occasionally to be found on man, but it is usually easy to trace the circumstances of contagion from definite mangy animals, whereas the disease of scabies which exists year after year in the poorer and more crowded parts of our cities is held to be due to the particular form—whether species, variety or race—known as *Sarcoptes scabiei* De Geer. Delafond and Bourguignon, indeed, state their opinion (1862, p. 102) that if human scabies depended entirely on successive generations from man to man it would have been stamped out long ago, because so easy to treat, but that it is reinforced from domestic animals; and again (p. 142) they say "Man seems rather to have the *Sarcoptes* and the Scabies of animals than a *Sarcoptes* and a Scabies peculiar to the human species." But whatever truth there may be in this, it is certainly the case that one form of *Sarcoptes*, in default of treatment, is perfectly at home on mankind, and can maintain its ground for an indefinite period without any reinforcement from other sources, and has come to be regarded as *par excellence* the *Sarcoptes* of man.

Incidentally it may be remarked that the same two investigators (D. and B.) insist on a remarkable difference between man and other animals, as the hosts of *Sarcoptes*. In other animals, they say, a certain "predisposition" is

necessary if the mite is to flourish, and the disease may often be cured by no other treatment than the supply of nourishing food and generally healthy conditions, whereas in man condition, age, sex, temperament, etc., are entirely unimportant. Indeed really sick, feverish subjects with serious inflammations do not furnish a favourable ground for scabies, which soon dies out upon them. Typhoid patients heal spontaneously during their fever, but on convalescence the scabies resumes its progress.

In the life of the male *Sarcoptes* four stages are recognised, egg, larva, nymph and adult. In the case of the female there are two nymphal stages, the second nymphal stage being remarkable inasmuch as it is ripe for fertilisation by the male but has not yet developed the orifice (tostome) by which the eggs are laid. This second nymphal stage is sometimes called the immature female (femelle pubère).

Description of *SARCOPTES SCABIEI*.

ADULT FEMALE. Length 330–450 μ ; breadth 250–350 μ . Body oval and testudiniform, being convex above and flattened below; of a translucent dirty white colour with the more highly chitinated portions brownish. The integument is finely striate over most of its surface, the striae being mostly oblique on the dorsal aspect, and transverse on the ventral surface.

There is no definite segmentation of the body, but it is nevertheless (in the living specimen) fairly clearly divided into two regions by a fold of the integument, the division being more distinct ventrally. These two regions are generally called the cephalothorax and abdomen, which is, unfortunately, an entire misuse of those terms as applied to other groups of Arachnida, as the posterior portion bears the last two pairs of legs. The terms notothorax and notogaster applied to these regions in their dorsal aspect are perhaps less objectionable. Mégnin (1895, p. 130) says that the body is divided into five incomplete segments by dorsal furrows, and Fürstenberg evidently is of the same opinion. The integument is furnished with an armature of cones, spines, scales and bristles which must be carefully studied since they are regarded as of great taxonomic importance.

Dorsal aspect. The fine parallel striae are for the most part absent on a large median posterior space (the posterior part of the notothorax and the whole of the notogaster) which is beset by about 140 minute conical scales, roughly arranged in transverse rows, and with their points directed backwards. Fürstenberg has figured these scales under a very high magnification and considers their particular shape characteristic of this species. They are mostly of the acorn-shaped variety. (Text-figure 1.)

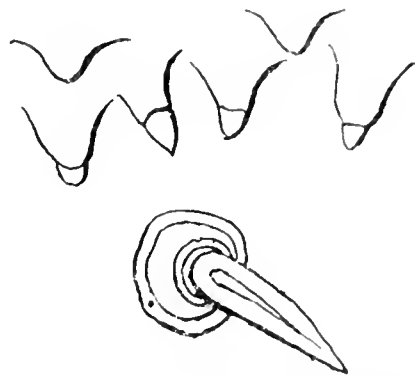
On the notothorax there is also a rectangular space free both from striae and scales but with a shagreened or rugose surface. This is the “clairière” of French and the “Blösse” of German writers, and it is the “plastron” or specially chitinated plate of Mégnin, who noted it first in the horse *Sarcoptes*, where its slightly yellow colour attracted his attention, but found it subse-

quently in all the varieties of *Sarcoptes* he was able to examine (*vide supra*). Munro (1919) figures, but does not mention, two small spots in front of the plastron which are probably Mégnin's "rudiments of stigmata." (Pl. XV, fig. 10.)

On either side of the notothorax, just posterior to this "plastron," there are three *notothoracic cones*, arranged in a triangle with its apex posterior. These "cones," which are in fact somewhat lancet-shaped, are articulated into the crater-like centre of circular low eminences.

On either side of the notogaster are seven backwardly directed spines, roughly arranged in two longitudinal rows, an inner row of four and an outer of three spines. These spines are of the same nature as the notothoracic cones, but more elongated. These spines, cones and scales are all calculated to render retrograde movement in its burrow impossible to the parasite.

The chaetotaxy, or arrangement of bristles, is especially important and none of the figures of the early investigators are accurate in this respect. Munro (1919), who both studied the literature on the subject and examined much fresh material, gives it as follows:



Text-figure 1. Dorsal scales and a notogastric spine of *S. scabiei* (after Fürstenberg).

On the notothorax there are four pairs of bristles:

(1) a pair "lying just below the camerostome." This appears to refer to the largest of three pairs of bristles which he figures as arising from the rostrum (see Pl. XV, fig. 10).

(2) a small pair close together near the anterior border.

(3) a pair of strong whip-like bristles, one on either side of the clairière or plastron, and reminiscent, as Munro says, of the pseudostigmatic organs of the Oribatidae. As a matter of fact it is quite common in various acarine groups (Gamasidae, Tyroglyphidae, etc.) to find strong bristles in this position, and it is possible that they have a special sensory significance—as they certainly have in the Oribatidae. When Fürstenberg, however, alludes to the "Tasthaar" he means the stronger of the two lateral hairs mentioned in (4).

(4) a pair of bristles (or hairs) on each side just anterior to the fold separating notothorax and notogaster, the anterior (and according to Munro's figure the more dorsally inserted) bristle being the longer. (This is Fürstenberg's Tasthaar.)

On the notogaster there is a pair of bristles on either side of the terminally situated genito-anal aperture, the inner bristle being somewhat longer than the outer.

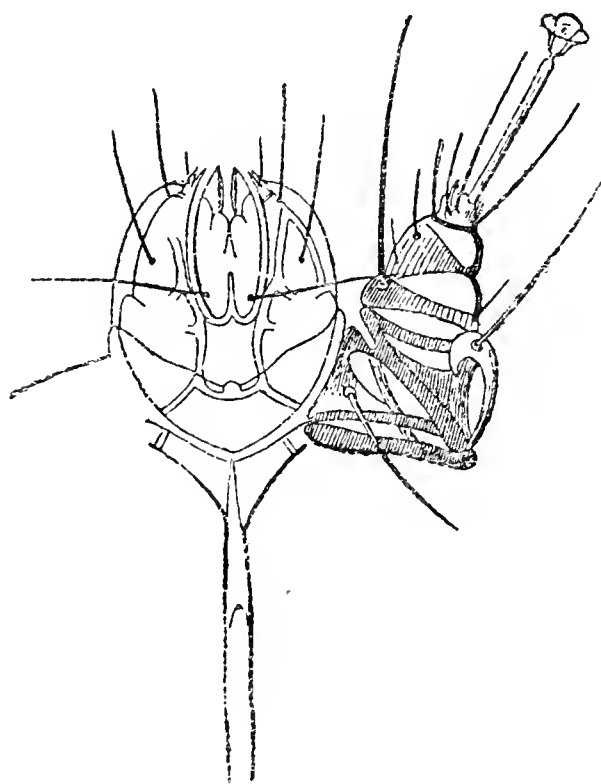
Ventral aspect. The rostrum and legs will presently be described in detail, but it is necessary here to mention the epimeres, which are striking features of the ventral surface, and have been considered of great taxonomic im-

portance. They are chitinous bars produced into the body from the skeletal tissues of the legs, and are arranged as follows:

From the first pair of legs the epimeres run inwards and meet just behind the base of the rostrum and are then produced backwards in a single sternal rod, ending in a fairly sharp point; the epimeres of the second legs are free and slightly curved, converging towards the sternal bar without reaching it, and ending at about the same level. Precisely how they terminate seems doubtful, whether in rounded knobs or in a slight fork. The epimeres of the third and fourth legs are short and curve towards one another till they nearly meet, but they do not fuse.

Immediately behind the end of the sternal bar is a transverse slit—the tocostome, or aperture for egg-laying, guarded by three very short bristles (see Pl. XV, fig. 11). There is also a pair of short bristles one on either side of the middle of the sternal bar, and another short pair between the fourth pair of legs.

The legs. The two anterior pairs of legs arise from the margin of the body and are larger than the third and fourth pairs, which arise from the under surface of the animal. Moreover the two



Text-figure 2. Rostrum and leg of *Sarcoptes scabiei* (after Railliet).

first legs terminate in *ambulacra* (ventouses), long tubular processes ending in a bell-shaped sucker, while these ambulacra are not found on legs 3 and 4, each of which is produced into a long bristle. Each leg consists of five articles (coxa, trochanter, femur, tibia and tarsus according to Dubreuilh and Beille), and has a strong chitinous skeleton, each article being strengthened by a chitinous ring, and there being also, apparently, an obliquely placed ring of chitin connecting the rings of the first two articles (Text-figure 2). The anterior legs have three claws, one (discovered by Mégnin as described above) on the inferior side of the trochanter, and two, of unequal size, at the extremity of the tarsus near the origin of

the ambulacrum. The ambulacrum itself is about as long as the whole limb. The legs are also furnished with a few bristles and some short spines (*piquants*).

The rostrum. The mouth-parts are enclosed in a sort of incomplete chamber, the *camerostome* formed by four prolongations of the integument—the epistome above, the cheeks (*joues*) on either side, and the hypostome (*lèvre*) below. They consist of six pieces, arranged in two rows; above are the chelicerae and pedipalpi, and below are the *mâchoires* (? labial palps) soldered to the sides of the hypostome and meeting posteriorly in a rounded plate, the *mentum*.

The pedipalps, which are above and slightly external to the "mâchoires" and just within the cheeks, are conical and three-jointed. Their chitinised base is contiguous to the epimeres of the first legs. The chelicerae are chelate and consist of a rod (tige) ending in two toothed processes, the inner of which is mobile. According to Dubreuilh and Beille (1895, p. 18) the dorsal edges of the chelicerae are in contact while their ventral edges are separated, so that there is between them and the hypostome a space, triangular in section, which is the mouth cavity. The chelicerae always act in alternation.

Delafond and Bourguignon (*Traité pratique*, p. 37) give a very interesting account of the mode of action of the mouth-parts. First the palps are separated and their sharp extremities plunged into the integument, which they loosen by repeated attacks. Then the chelicerae come into play, breaking down the adhesions "between the corneous and mucous layers," and acting alternately. Now the muscles which actuate them pass through a narrow orifice to the thorax, and this would result in pulling the left mandible to the right, and the right to the left. This is prevented by a small horny plate which separates the mandibles behind and turns on a fixed vertical axis.

ADULT MALE. The male differs from the female in the following respects. It is much smaller, measuring $200\text{--}235\mu$ in length by $145\text{--}170\mu$ in breadth, and the fourth pair of legs terminate, like the anterior legs, in ambulacra. The epimeres of the second pair of legs are forked at their extremity¹, and the epimeres of legs 3 and 4 are not only fused together at each side, but the two pairs of epimeres are united by a transverse bar from the middle of which a chitinous structure in the form of an inverted Y proceeds backwards and constitutes the *epiandrium* or genital armature, for the genital aperture of the male lies between the fourth pair of legs, while the anus, as in the female, is terminal.

The dorsal aspect presents three "plastrons" or "clairières," one on the notothorax and two on the notogaster. That on the notothorax is much larger than in the female, and is longer than broad. Those on the notogaster are small and subcircular and lie on either side of the posterior end of the abdomen.

The immature stages. There appears to be no detailed account of the structure of any of the immature stages of *S. scabiei*. Munro thus describes the second nymphal or immature female stage (femelle pubère).

"Length $220\text{--}250\mu$; breadth $170\text{--}200\mu$; rugose area (plastron) rectangular; dorsal scales numerous; bare area notogastric, small; notogastric spines twelve in number; third and fourth posterior legs ending in bristles; epimeres of anterior pairs of legs (? the second pair) forked. Tcostome and tocostomal hairs absent."

The first nymphal stage, according to Dubreuilh and Beille (p. 20) had

¹ Fürstenberg maintains that in this species the epimeres of the second legs do not end freely but are joined by a transverse bar of chitin just anterior to that connecting the epiandrium with the epimeres of the posterior legs (*Krätzmilben*, Pl. I, fig. 8).

never been observed in the human *Sarcoptes*, but Munro found it on two occasions, and describes it as follows:

“Length 160μ ; rugose area indistinct; dorsal scales numerous, bare area well defined; notogastric spines twelve in number. Fourth pair of legs ending in bristles which are shorter than those of the third pair; only one pair of anal bristles present. Epimeres of the anterior pairs of legs forked.”

Dubreuilh and Beille (p. 20) say that the nymphs are of two sizes (measurements not given) and that the smaller produce males and the larger females.

The larva is hexapod, the last pair of legs ending in bristles. Its length is from 110 to 140μ and its breadth 90 to 110μ . Rugose area ill defined; dorsal scales indistinct except at the sides so that a large bare area is present; notogastral spines ten; only one pair of anal bristles.

The characters which have generally been relied on to separate the various forms which have been regarded by some authors as distinct species and by others as varieties are size, shape, the rugose areas, the epimeres, and the number and particular pattern of the various spines, scales, etc., which go to make up the dorsal armature. It has to be noted that the limits of size in a single form like *S. scabiei* are very considerable, and that the shape is necessarily rather indefinite in animals so feebly chitinised, while the other characters are so minute and so difficult of observation that no two writers are absolutely in agreement in their description of any one variety. It would seem to be the first task of a new investigator to examine minutely the human sarcopt in its various stages and to determine authoritatively the details of its structure, verifying or rejecting the statements of previous writers. When this is once accomplished the ground will be cleared for a comparison between it and the forms which affect other mammalian groups.

Life-History of the *Sarcoptes* of Man.

Excavation of the gallery. Immediately after her last moult the female begins to burrow, showing a distinct preference for certain regions. If confined under a watchglass she may be caused to burrow anywhere, but only sufficiently to bury herself. If free to wander, she selects either the interdigital spaces, the wrists and their ulnar margin, the elbows, the anterior folds of the axillae, the pubic area, the buttocks, the back of the knee, the ankle or the toes.

Holding on by the suckers of the anterior legs she raises the hind end of the body on the bristles of the posterior legs till her position is almost perpendicular, and commences to cut into the skin. She can completely bury herself in $2\frac{1}{2}$ minutes (Munro, p. 15). When concealed, she may either remain inert for a time or continue burrowing, and this depends somewhat on the temperature, which, according to Delafond and Bourguignon, must be between 10° and 30° C. Normally the burrowing only occurs at night, when the patient is warm in bed, but it may be induced by artificially warming the place where the parasite has buried itself. Munro (p. 15) found that the average length of

burrow daily excavated was between two and three millimeters. Török and Dubreuilh have shown that the galleries are entirely confined to the corneous layer.

A fairly constant characteristic of *Sarcoptes* burrows is the presence of vesicles (vésicules perlées), minute translucent indurated swellings, which are regarded as second only to the galleries themselves in diagnosing scabies. It was these vesicles which misled investigators at the beginning of the 19th century, for they sought in vain for the mite in their interior—though Moffett had stated as early as 1634 that it was not to be found in them, but in their neighbourhood. The vesicle, indeed, forms *below* the gallery, a short distance behind the burrowing mite. The gallery and the vesicles seem to be directly attributable to the *Sarcoptes*, but the other complications which so frequently attend severe scabies must be regarded as secondary.

Oviposition. The mite deposits eggs in groups of 2–4 (Gerlach says 3–6) as she proceeds with her burrowing, resting after each act of deposition. Black masses of excrement are found in the neighbourhood of the eggs. The eggs are oval, $150 \times 100\mu$. They appear to be laid in a varying condition of development, which may partly account for the very varying incubation periods allowed by different writers. Bourguignon gives ten days and Fürstenberg seven days, but Gerlach states that this is certainly inaccurate, and that the usual period is 64–76 hours. Munro agrees with the latter estimate when nothing has happened to delay matters, but he says that partially developed eggs may have their development arrested by unfavourable conditions and afterwards resume it.

Unless disturbed the female makes a single burrow and dies at the end of it. The burrow is always sinuous, and just the width of the mite. It may attain 3 cm. in length. At intervals there are holes in the roof—to admit air or for the egress of the larvae and nymphs. The number of eggs is doubtful—possibly because few burrows are entirely undisturbed. The largest number actually found in a burrow seems to be 21, but there exists a pretty general belief that the female lays in all about 40 or 50 eggs.

The larvae. Several authors (Hebra, Railliet, Dubreuilh) state that the larva moults three or four times. Munro found no evidence of this. They live in the burrows and bore into its floor, or emerge and burrow deeply into the skin outside—more deeply, according to Gerlach, than the females themselves.

The nymphs. Nothing has been observed as to the habits of the first-stage nymph. The second-stage nymph (femelle pubère) makes a burrow like that of the adult but smaller, and devoid of vesicles. These short burrows have lateral branches or pockets excavated by the male (Munro, p. 22).

Mégnin (1893, p. 132) says that he has found the human *Sarcoptes* on small house-dogs, and very frequently on ferrets, and that Railliet has found it on rabbits.

The above is a condensed account of the structure and life-history of the human *Sarcoptes* as at present known, and it is now proposed to deal briefly with the forms which are generally considered distinct from it. In order not to prejudge their status it will be as well to allude to them simply under the names of the animals they affect, leaving open the question as to whether they are species, varieties, or merely races. The characters which are supposed to distinguish them from the human *Sarcoptes* will be indicated, and anything else of importance to our purpose will be added.

A good plan will be to give the *Tierreich* definition of each form at the outset. It aims at the briefest possible characterisation, and provides a convenient starting point.

Many of these forms have been naturally and artificially communicated to different animals from their proper hosts, and the matter will be referred to in due place, but in this connection Delafond and Bourguignon utter a warning which is very much to the point. Artificial attempts to communicate scabies from one animal to another frequently fail, but too much importance must not be attributed to such failure. Probably in nature there are many abortive attempts at invasion of another animal before success is attained, and in artificial infection the conditions are generally much less favourable. Comparatively few mites are transferred, and many of these may be unsuited, from their nature (*e.g.* unfertilised nymphs) or from their condition through injury in the act of transference, to establish the disease on an animal which might very well succumb to repeated natural invasions.

SARCOPTINAE PARASITIC ON OTHER ANIMALS.

Scabiei-crustosae.

Tierreich: Fürstenberg, 1861.

Dorsal scales blunt; no rugose area; notogastric spines long and pointed. The epiandrium reaches the epimere.

♀ 410 × 340μ.

♂ 170 × 150μ.

Man; causing Norwegian itch. Norway, Germany, France.

Fürstenberg's description is in substance as follows:

Female. Body nearly round, not much longer than broad, the fourth thoracic segment prominent at the sides, the sensory hair borne on it being very long¹. Back with scale-like prominences surrounded by a rim of chitin. Six long notothoracic cones and 14 curved, very sharp notogastric spines.

Male. Body nearly round; fourth thoracic segment rather prominent, with strong, long sensory hair. Very few dorsal scales, at the edge of the thorax and abdomen. A shield-like area (*Abgrenzung*) finely punctate and

¹ This is the third dorsal hair, the more anterior of Munro's two lateral hairs. Fürstenberg believed that folds—often conspicuous at the sides—indicated primitive segmentation.

rounded behind, extending from the head to the fourth thoracic ring. Epiandrium firmly fused with epimere.

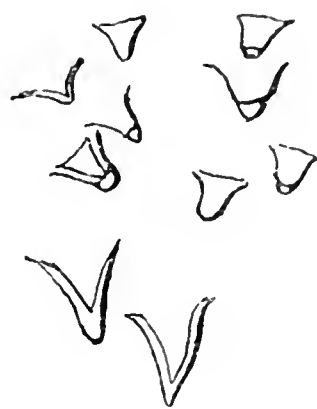
This *Sarcoptes* is the cause of "Norwegian itch" in man, and lives in innumerable superimposed burrows in the crusts. Chaetotaxy like that of the common human *Sarcoptes*, but the folds of the body are more marked. ♀ $415 \times 341\mu$. The head is comparatively large.

The male is rather smaller than that of the common human *Sarcoptes*— $172 \times 153\mu$. (Fürstenberg, p. 212, Pl. V.)

The disease of "Norwegian itch" was (according to Railliet) first recorded by Boeck and Danielssen in 1848. It is characterised by gigantic crusts. Sometimes these arise at the outset, but more often they do not appear for several years (3–9). Callosities 1–6 mm. in thickness appear on the palms of the hands, the soles of the feet, the wrists, elbows and knees. The scabies may extend to the face and the hairy scalp.

Mégnin thinks this parasite identical with *lupi*, but Railliet considers them too unlike.

Fürstenberg considered the peculiar severity of the disease due to the particular *Sarcoptes* at work, but Hebra attributes it to special susceptibility of certain subjects, and considers the parasite to be nothing but the common human *Sarcoptes*. It has been noted not only in Norway, but in France, Austria, Germany, Denmark, Russia and Turkey.



Text-figure 3. Examples of dorsal scales of *S. scabiei-crustosae* (after Fürstenberg).

Lupi.

Tierreich: Mégnin, 1880.

Dorsal scales pointed; no rugose area; notothoracic cones long and blunt. ♂ very elongate (langgestreckt). Epiandrium firmly united to epimere.

♀ $370-400\mu \times 280-300\mu$.

♂ $270 \times 160\mu$.

Canis lupis. France.

Mégnin's measurements are:

♀ $400 \times 280\mu$. ♂ $270 \times 210\mu$.

He found it in 1875 in four young wolves (at the Muséum de Paris) which had their hides covered with thick, yellowish, moist crusts, nearly a centimeter thick in places. Transferred to a horse, they gave rise to a scabies of exactly the same nature—even to the characteristic smell. Mégnin considered it the cause of "Norwegian itch" (*vide ante*) but his view is not universally accepted.

Equi.

Tierreich: Gerlach, 1857 (von Hering, 1838).

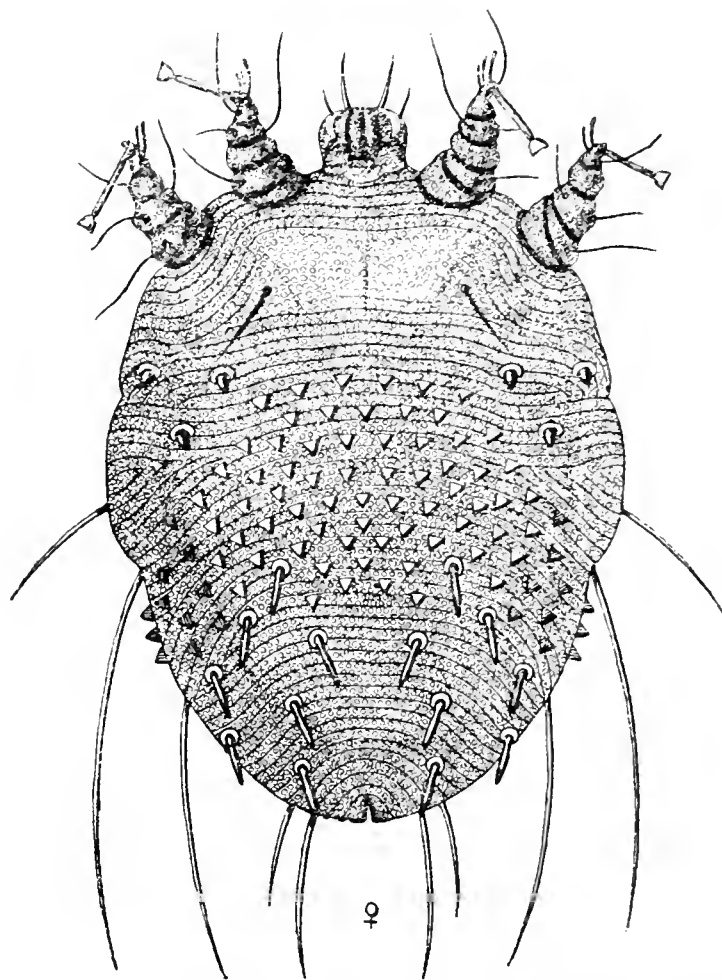
Dorsal scales small, scarcely longer than broad; an anterior and a posterior rugose area; notogastral spines blunt; notothoracic cones very short; epiandrium scarcely reaches the epimere.

♀ 450–500 μ \times 310–370 μ .

♂ 230–280 μ \times 150–200 μ .

Horse, but can flourish on ass, mule and man for a short time.

This form was independently discovered by Gerlach and Delafond simultaneously. It is the cause of “dry mange,” “symptomatic mange,” or “epizootic mange” in the Equidae (Gerlach, 1857, p. 116, Pl. II, figs. 8–10).



Text-figure 4. *Sarcoptes equi*, ♀, according to Railliet.

Gerlach says the ♀ is somewhat longer and narrower than the human *Sarcoptes*, but is only to be distinguished when seen in the bulk; his measurements of the ♀ (reduced from fractions of an inch to microns) are 440 \times 300 μ .

Delafond and Bourguignon remark that the “solipèdes” have two mange parasites, a *Sarcoptes* and a *Psoroptes*, the former transmissible to man, the latter not. (There is also a Chorioptic scabies of the horse.)

Neumann (p. 131) says “Sarcoptic scabies begins most frequently at the withers, and extends to the sides of the neck, shoulders, back and sides. It does not easily invade the extremity of the limbs, and it respects the parts covered with strong hairs. The Psoropt, on the contrary, appears to seek the parts avoided by the Sarcopt.”

There is first pruritus, then depilation, then formation of crusts. Many experiments have been made on the transmission of *Sarcoptes* from other animals to the horse, and of the horse *Sarcoptes* to other animals. Delafond and Bourguignon several times succeeded in transferring the human Sarcopt to the horse, where it burrowed galleries, but always died out after a short time. They also describe a case in which horses groomed with brushes and sponges which had been used to clean mangy lions contracted the disease—which was artificially cured. According to Neumann (p. 133) Wallraff reports a case of horses contracting scabies from mangy goats.

There are many cases of the transference of the horse Sarcopt to man (*e.g.* Delafond and Bourguignon, *Traité pratique*, p. 295). It usually disappears spontaneously in from 15 days to 6 weeks, or at all events easily yields to treatment.

Mégnin (1893, p. 144), in May 1892, investigated an extraordinary case of very severe crusted scabies in man and found the sarcopt to be that of the horse. The patient had had charge of a horse hauling barges on the Oise.

Neumann (p. 134) says "Sarcoptic scabies of the horse appears to be capable of transmission to the bovine species, though up to the present time no one in practice has observed this form of mange in cattle. The possibility of this transmission rests on facts published by Robert Fauvet and Grogner."

The following results of experiments by Delafond and Bourguignon on the longevity of the mite may be given here.

(1) 10 ♂, 10 larvae and 10 ♀ in a glass tube covered with paper, in a room at temp. 10°–15° C.

larvae died from 4th to 6th day,
 males ,, ,, 5th to 8th ,,
 females ,,, ,, 6th to 9th ,,

(2) Experiment repeated in a stable at temp. 15°–20° C.

larvae died from 5th to 6th day,
 males ,, ,, 6th to 8th ,,
 females ,,, ,, 9th to 10th ,,,

(3) When kept in tubes containing hairs and fragments of crust and litter, and moistened from time to time (at 15°–20° C.)

larvae lived 10 days,
 males ,, 13–14 days,
 females ,,, 14–16 ,,

Gerlach kept the mites alive three or four weeks in a piece of mangy skin kept moist.

Hering's *S. equi* was the *Psoroptes* of the horse.

Canis.

Tierreich: Gerlach, 1857 = *squamiferus* Fürst. (in part).

Dorsal scales hardly longer than broad; no rugose area; notogastral spines long and slender; anterior arm of epiandrium feebly connected with epimeres of legs 3 and 4.

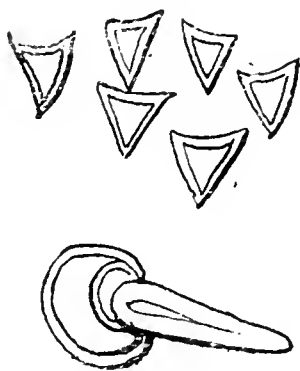
♀ 300–450 μ \times 230–350 μ .

♂ 190–250 μ \times 160–180 μ .

Dog, appears to be communicable to man but not to other animals.

There is great confusion with regard to the dog *Sarcoptes*. This animal is, of course, subject to a Demodicic mange, but several authors record a Sarcoptic scabies of the dog, though no two writers agree about it.

Gerlach's *canis* is "smaller than the human species with legs of a darker yellow-brown colour," but he describes and figures (Pl. II, fig. 11) the ♀ with two posterior rounded projections though the male has no corresponding "cylinders." His measurements of the ♀ are 300 \times 225 μ . Now he described at the same time a form from the pig which he called *suis*, the female of which



Text-figure 5. Dorsal scales and a notogastral spine of *S. canis* (*squamiferus*) (after Fürstenberg).

had no such projections, but the male of which is described and figured with two "cylinders" near the generative opening (Pl. III, fig. 16). Fürstenberg (1861) considered these two forms the same, and fuses them under the name *squamiferus*, but he omits the projections on the abdomen of Gerlach's ♀ *canis* and the "cylinders" of Gerlach's ♂ *suis* (Fürstenberg, p. 214, Pls. II and IV). Delafond and Bourguignon (1862) threw doubt on Gerlach's *canis* because of the abdominal projections, which, they alleged, would remove it from the genus *Sarcoptes* altogether, but they knew of a dog *Sarcoptes* which Bourguignon had described in 1853 (thesis for a prix de Montyon), which was "notably smaller than the human *Sarcoptes* (♀ 300 \times 250 μ , ♂ 200 \times 160 μ) but had the same form."

On the other hand Canestrini (*Prosp. Acarofauna Italiana*, vi. p. 741, Pl. 63) describes and figures the Sarcopt from a Neapolitan dog, the female measuring 450 \times 350 μ .

Delafond and Bourguignon (1862) performed many experiments with the *Sarcoptes* they found on the dog. Healthy dogs confined with mangy dogs caught the disease. Crusts placed on unhealthy dogs communicated it in a severe form. A dog powdered with the mites from the skin of another dog which had died of mange and then badly fed and housed, contracted scabies which was allowed to go to extreme lengths, but there was spontaneous cure on the resumption of good feeding and housing. This experiment was repeated.

Conditions of age and race are important. Long-haired dogs favour sarcoptic mange, as do masterless or ill-tended dogs and chained dogs with damp kennels. Good housing is of the utmost importance.

Sarcoptic mange of the dog is alleged by different writers to be not uncommon, but investigators have generally had a difficulty in finding it. It usually begins on the head, muzzle, round the eyes and on the ears, but may appear on any part of the body (*Traité pratique*, p. 184 *et seq.*).

In any new investigation this form must be considered in connection with that obtained from the pig which is next discussed.

According to Neumann (1905, p. 183) the dog is also subject to the form *lupi*.

Besides a Demodicic mange, and scabies due to the *Sarcoptes* above mentioned, a Chorioptic mange may be found on the dog.

Suis.

Tierreich: Gerlach, 1857 (*squamiferus* Fürst. in part).

Dorsal scales longer than broad, pointed; an anterior rugose area; notogastral spines long, slender, pointed; epimere of legs strongly curved outward; epiandrium reaches the epimere; body nearly globular.

♀ 350–500 μ \times 290–390 μ .

♂ 250–350 μ \times 190–300 μ .

Sus domesticus. Transmissible to man. Germany, France, Italy.

Gerlach (1857) says that Gurlt had found it many years previously on wild boars, and since then Hertwig and Gerlach had recorded it from wild swine. Gerlach's measurements of the ♀ (reduced from fractions of an inch to microns) are 380 \times 270 μ (Méglin gives the measurements ♀ 500 \times 360 μ , ♂ 320 \times 290 μ , and Neumann gives ♀ 400–500 μ \times 320–390 μ , ♂ 250–350 μ \times 190–300 μ). Gerlach describes it as very like that of man, and that of the horse, between the two in size, and conspicuously broader in front and narrower behind, even discounting the fact that the abdominal width varies according to the contained eggs. He, however, gives the ♂ two genital "cylinders" (Gerlach, Pl. III, fig. 16).

Delafond, in 1857, found on the domestic pig a *Sarcoptes* which he considered identical with that of man, but Méglin, who has examined Delafond's microscopic preparations of it, says it is Gerlach's *suis* (Méglin, *Ac. parasites*, p. 140). Fürstenberg considers Gerlach's *suis* and *canis* identical, and re-describes them as *squamiferus* (see *canis*).

Méglin thought the pig had two *Sarcoptes*—a large form on the body and a small form in the ears. The latter, whose existence has been disputed, is next dealt with under Canestrini's name *parvulus*. The ordinary pig sarcopt first occurs on the head—chiefly the ears and round the eyes; then the withers croup, and inner surface of the thighs. Later, the whole body. No galleries are observed, but when advanced there is abundance of crusts.

It is transmissible to man and to the dog, but soon dies out. This is the largest known *Sarcoptes*.

Parvulus.

Tierreich: Canestrini, 1894.

Body (Rumpf) very small.

♀ $288 \times 216\mu$.

♂ $168 \times 128\mu$.

In outer ear passage of Sus domesticus.

This species (or variety) is merely nominal, the name having been given in 1894 by Canestrini to a very small form found by Guzzoni in 1877 in the ears of a pig. Gurlt and Spinola, Gerlach, Mégnin and Railliet (1895, p. 635) appear to have come across the same form, but there is no description of it. Neumann (1905, p. 177) states definitely that there is no reason to believe in the existence of a second pig sarcopt, but he does not explain the great difference in size between the particularly large form usual on the pig and the minute form alleged by several observers to occur in the ears of that animal.

Furonis.

Tierreich: Railliet, 1893.

Dorsal scales dense (dicht), slender (fein); an anterior rugose area; epian-drium firmly united to epimere.

♀ $330-420\mu \times 270-300\mu$.

♂ $210-220\mu \times 160-180\mu$.

Mustela furo. France.

The measurements are those given by Railliet (1895, p. 656), who says the *Sarcoptes* was first described by Peuch in 1869, and that it is very common on the ferret, which probably contracts it from rabbit burrows frequented by polecats. It principally attacks the head and legs, but sometimes invades all the surface, causing brown or yellowish crusts. On the feet, the crusts especially occur on the plantar surface and at the base of the claws, which often become abnormally long and turned upwards. Peuch, though he made many attempts, did not succeed in transmitting it either to man or to the dog.

Peuch's account was published in 1869 and this I have been unable to consult, but it seems to be chiefly clinical, and the meagre details obtainable are those furnished by Railliet (1895, p. 656). He calls the dorsal scales *minces et serrées*, and says that the rugose area is faint.

Cuniculi.

Tierreich: Neumann, 1892 (*Rev. Vét.*, March, 1893).

Dorsal scales numerous, sharp, as long as broad; no rugose area; notogastric spines broad at the base, bluntly pointed; epimere of leg 2 of ♀ strongly bent outwards; front arm of epiandrium rudimentary, not reaching the epimere.

♀ $410-440\mu \times 320-340\mu$.

♂ $230-250\mu \times 170-180\mu$.

Lepus cuniculus. France, Italy.

Neumann's paper has been unobtainable, and he does not describe this *Sarcoptes* in his *Parasites*. Railliet (1895, p. 656) says: the dorsal scales of the ♀ are not very sharp but rather strongly chitinised, at least in front and at the sides. A posterior "clairière." Epiandrium rather loosely connected with epimere. ♀ $340-400\mu \times 260-300\mu$, ♂ $210-228\mu \times 155-178\mu$. Oviparous or viviparous.

Its habits seem to be precisely like those of *furonis* in the ferret. It affects in turn the nose, lips, chin, the base of the claws, and the plantar surface of the feet, sometimes extending to other parts, and causing greyish crusts. The disease is very contagious among rabbits and often ends fatally in a few weeks. Neumann did not succeed in communicating it to dog, sheep, ox, pig or horse, but Railliet succeeded in the case of the capybara and the ferret.

The question naturally arises as to whether this form is distinct from *furonis*. The chief differences seem to be its larger size, and the connection between the epiandrium and the epimeres of legs 3 and 4. The rugose area might well escape observation.

Canestrini calls this form *S. praecox*, considering *cuniculi* preoccupied for *Notoedres* (*vide infra*). He gives (*Prosp. Acarof. It.*, VI. Pl. 69) a figure of the ♂ showing the trochantal claws on leg 1—alleged by Mégnin to be always present in *Sarcoptes*.

Ovis.

Tierreich: Mégnin, 1880.

Dorsal scales not numerous; an anterior and a posterior rugose area (or clear space) not very extensive (ausgedehnt). Epiandrium feebly connected with epimere.

♀ $314 \times 300\mu$.

♂ $220 \times 160\mu$.

Ovis aries. France, Italy.

Delafond (1858) was the first to find this parasite, which, of course, must not be confounded with the cause of "sheep scab" (which is a *Psoroptes*), or with the *Chorioptes* which also affects this animal. It causes "black muzzle" or "face mange," and is much less common than the *Psoroptes*.

It only attacks parts free from wool, first appearing on the upper lip and round the nostrils, and later invading the face, forehead, cheeks and eyelids, and sometimes the intermaxillary space. When of long standing it may appear between the fore-legs, on the belly, and round the joints.

Delafond's subjects were Neapolitan sheep brought to Paris. Both the *Psoroptes* and the *Sarcoptes* were present, and a pupil (Polvent) at the Alfort Veterinary College who treated them became badly infected by the *Sarcoptes*, which Delafond considered to be exactly like that of man. The pupil, though in a terrible condition, persisted for 49 days without treatment, but was then so ill that cure was undertaken. This occupied a fortnight.

The *Tierreich* measurements are those given by Mégnin.

Vulpis.

Tierreich: Fürstenberg, 1861.

No rugose area; notogastral spines long and pointed; notothoracic cones longer than usual; anterior arm of epiandrium well developed.

♀ $442 \times 315\mu$.

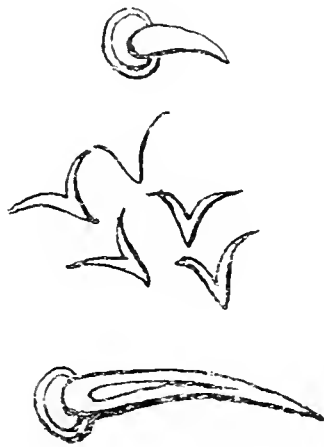
♂ $245 \times 185\mu$.

Canis vulpes.

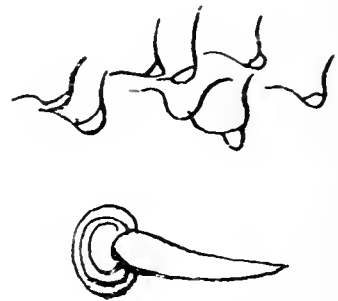
Fürstenberg's description is in effect as follows:

Female. Body long-oval, testudiniform; dorsal scales in rows; notothoracic cones rather long, curved, with the points bent backwards and downwards. Notogastric spines long, not very strong, slightly curved, and apparently sharp-pointed. $442 \times 315\mu$.

Male. Body nearly round, not much longer than broad; a few dorsal scales, only at the boundaries of the thorax and abdomen; otherwise with the armature of the ♂ of *Scabiei-crustosae*. $245 \times 185\mu$.



Text-figure 6. Notothoracic cone, dorsal scales, and notogastral spine of *S. vulpis* (after Fürstenberg).



Text-figure 7. Dorsal scales and a notogastral spine of *S. caprae* (after Fürstenberg).

The female, though inferior to the human *Sarcoptes* in size, resembles it in general appearance. The bulging of the fourth thoracic segment is not so marked as in *Scabiei-crustosae*, and the sensory hair is only of medium size.

The disease it conveys is like that of *Scabiei-crustosae*, causing crusts often half-an-inch thick.

The specimens were from a mangy fox, the tail of which was sent to Fürstenberg by his brother in 1857 from Gagen in Rügen. It arrived three days after the animal was killed, but the mites were still alive, and continued to live three or four days more (Fürstenberg, p. 213, Pl. VI).

Railliet (1895, p. 659) says that Walz first recorded this mite in 1809.

Caprae.

Tierreich: Fürstenberg, 1861.

Dorsal scales blunt (stumpf); a posterior faint rugose area; epiandrium feebly connected with epimere.

♀ $345 \times 342\mu$.

♂ $243 \times 188\mu$.

Goat, sheep, horse, ox and man.

The main points of Fürstenberg's description are these:

Female. Body nearly round, the thorax broader than the abdomen, the folds at the side moderately deep. Dorsal scales short, ending in a terminal chitinous portion which is generally rounded, sometimes pointed—only occasionally nail-like (genagelte) scales. Notothoracic cones long, acorn shaped; notogastric spines fairly long and apparently sharp-pointed. $345 \times 342\mu$.

Male. Oval, nearly egg-shaped, a few marginal dorsal scales. $243 \times 188\mu$.

They live in the skin and crusts of Egyptian pygmy goats (Zwergziege). The specimens were sent by Prof. Müller of Vienna (Fürstenberg, p. 214, Pl. VII, figs. 72–79).

The disease chiefly affects the head and ears, then the trunk, and lastly the limbs. It is often epizootic with considerable mortality. Wallraff recorded a case in Grisons during the years 1851–4. In the spring of 1853, out of 2596 goats 1015 had developed mange and 250 had died. At the end of the attack 500 goats had been lost.

Henderson recorded it in 1851 from a Persian goat in London. Except for the Swiss cases it has always been found in African or Asian varieties of goat.

It seems readily transmissible to other animals, and in the Swiss epizootic horses, oxen, sheep, pigs and especially man caught the disease from the goats, and had it in a severe form. In Henderson's case it passed to the horse and thence to man.

Delafond and Bourguignon (1857) say: "this species is remarkable for the size of its ambulacra (ventouses) on the anterior legs, and for the length of its posterior hairs."

Hebra found no difference between this *Sarcoptes* and that of man.

The goat is also subject to a Chorioptic scabies.

Leonis.

Tierreich: Canestrini (the "Sarcopte du lion," Delafond and Bourguignon, 1862).

Dorsal scales longer than broad; notogastral spines long, slender, pointed; notothoracic cones short, blunt, swelling in the middle; epiandrium feebly united to epimere.

♀ $460 \times 350\mu$.

♂ $250 \times 180\mu$.

Felis leo. Ménagerie Pianet, France.

Canestrini (*Prosp. Acarof. It.*, vi. Pl. 64) figures this sarcopt from preparations sent him by Trouessart. They were prepared by Neumann, and bore the label "*Sarcoptes scabiei* du lion, Ménagerie Pianet, 3, v. 92." The ♀ shows remarkable dorsal projections of the armature of the first legs.

The long account given by Delafond and Bourguignon (1862, pp. 229–233) remains our chief source of information about this *Sarcoptes*—which, however,

was considered by those authors identical with that of man. Borelli brought to Paris in his menagerie five terribly mangy lions, from which five assistants contracted severe scabies. Less severely, six horses and three grooms were affected, and in addition a bear and a hyaena, after holding out as long as their general health was good, lost condition and then quickly succumbed.

Delafond and Bourguignon attempted to transmit the disease to rabbits and guinea-pigs, without much success. They conclude that this form of scabies is particularly severe, nearly always proving fatal in a short time, but that it requires a predisposition on the part of the animal attacked.

Dromedarii.

Tierreich: Gervais, 1841 (*Ann. Sci. Nat.*, Ser. 2, xv. p. 9).

Dorsal scales longer than broad, sharp-pointed, numerous; no rugose area; notogastral spines long, narrow, rather blunt at the end; notothoracic cones, twice as long as they are broad at the base. The epiandrium is not connected with the epimere.

♀ 360 × 330μ.

♂ 290 × 180μ.

Camel, llama, giraffe, Antilope bubalis—not m...

Canestrini (*Prosp. Acarof. It.*, vi. Pl. 62) figures the ♂ and the larva of this form. The trochantal claws appear to be very conspicuous.

Gervais obtained his material from a mangy dromedary brought from Africa to the Jardin du Roi, and killed as soon as its condition was recognised. He deals in the paper cited with the *Psoroptes* of the horse, the *Sarcoptes* of man and the present form, and gives figures. He says the two *Sarcoptes* so closely resemble each other as to be easily confused without close examination. "One might even suppose that it is to this similarity of organisation that it owes the power of passing with such facility from the animal to which it is proper to man, and of transmitting the disease from one to the other."

It appears, therefore, that the *Tierreich* account is wrong as regards man.

Gervais proceeds: "The form is almost the same, but the *Sarcoptes* of the dromedary is a little more elongate than that of man; the papilliform tubercles of the back have not quite the same disposition; in the human species the bilateral hair is larger and more recurved, and the inner pair of posterior hairs are the longer" (whereas in *Dromedarii* they are the shorter).

He adds some obscure statements about the epimeres, and says that the dromedary *Sarcoptes* is larger, "which no doubt accounts for the more intolerable pain it inflicts when it attacks man."

The four "doubtful species" of the *Tierreich* are little more than names, taken from the animals on which the scabies was observed, and they may be rapidly dealt with. They are *aucheniae*, *hydrochaeri*, *rupicaprae* and *wombati*.

Aucheniae.

One among a number of llamas, sent to France in 1858 and deposited at the Muséum de Paris, became mangy and was sent to the school at Alfort, where Delafond and Bourguignon examined it. The following year there were four more cases. The *Sarcoptes* gives rise to a scabies which becomes very rapidly generalised and forms large greyish crusts, very hard and adherent. The disease was contracted by two pupils who handled the animals, and they were so severely attacked that treatment was necessary after a month. Delafond and Bourguignon (1862, p. 383) say that the parasite is "like that of man, the dog and the lion," but add that "the male has, as special character, a short, slender, sharp appendage on the outer side of the ambulacrum."

Railliet (p. 634) says that the dorsal scales leave no "clairière," and that the measurements are ♀ $340 \times 264\mu$, ♂ $245 \times 182\mu$.

Hydrochaeri.

Found on *Hydrochaerus capybara*, the Capybara, Cabiai, or Carpincho of S. America and the W. Indies—the largest living rodent.

Mégnin (1893, p. 132) gives its measurements as ♀ $357 \times 300\mu$, ♂ $220 \times 160\mu$.

Neumann (1905, p. 19) says it attacks ferrets, and he apparently thinks it identical with *furonis*. †

Rupicaprae.

According to Fürstenberg (p. 67), Hering (p. 603) says this *Sarcoptes* is roundish, narrowed posteriorly, irregular (höckerig) at the side, almost hairless, but the description is confused. Delafond and Bourguignon, judging from Hering's description and figures (which I have not seen), say that it is notoedric.

There is further confusion by Hering's statement that the female has two roundish projections, and the male a pair of suckers—which would remove it from the Sarcoptinae altogether.

Wombati.

There seems to be no description of this form. Duméril found it in the skin, covered with crusts, of a mangy wombat brought from Australia to the Muséum de Paris, and Fournier pronounced it to be identical with that of man. The keeper, and his assistants who prepared the skin, contracted an intense scabies with vesicles larger than those of the common scabies of man.

SARCOPTES ON CATTLE.

Robin in 1860 announced the discovery of a sarcopt on the ox, but the existence of a form proper to the bovidae has until recently been questioned and none has been described under the title *bovis*. Most of the old records of scabies in cattle were cases attributable to contagion with mangy animals

of other species—horses, cats or goats. Cases are, however, quite frequent, at all events in the British Isles, of a sarcoptic disease in cattle where no such origin can be traced, and until further investigation it must be held that a form of *Sarcoptes* exists with quite as sound a claim to the specific or varietal name *bovis* as have the other forms found on domestic animals to their respective titles.

Though the ox *Sarcoptes* has not been described and named, its occurrence has been frequently noted within the last 20 years, and there are at least two figures of it. M'Fadyean (1900, p. 73) gives an illustration of it which he states (in a footnote, *Ibid.*, XIII. p. 79) is a microphotograph, showing that it is an obvious *Sarcoptes*, and apparently proving that it possesses the unusual characteristic of three pairs of anal hairs. The second figure, also a microphotograph, is given by Williams (1917, p. 77), but the anal hairs are not distinct. I find, indeed, that the existence of a bovine sarcopt is no longer considered doubtful by veterinary surgeons, and that some economic entomologists are acquainted with it. It seems to be quite common in the north of England and in Scotland, and my friend Dr Stewart McDougal goes so far as to say that if he requires *Sarcoptes* for teaching purposes he finds cattle the most convenient source of material. It is clear that any investigation undertaken to determine the affinities between the sarcopt of man and those of domesticated and wild animals cannot afford to neglect the hitherto undescribed form which affects cattle.

Among the scattered and fragmentary records which possibly concern this sarcopt may be mentioned that of Bieler (1892) of Lausanne. He contracted a very severe form of scabies through carrying in his pocket, wrapped in paper, some hairs taken from a mangy bison.

NOTOEDRES Railliet 1893.

The *Tierreich* definition is:

Male without anal cylinders, ♀ without copulation tubes. Long unjointed ambulacra on the two anterior legs of ♀ and on the two anterior legs and the second posterior leg of the ♂. Anus dorsal.

Oviparous parasites of mammalia.

Mégnin (1893, p. 145) says that *N. minor* differs from *S. scabiei* by smaller size, rounder body not showing any folds or constrictions, dorsal anus, and concentric undulating ridges in place of dorsal scales.

There appear to be two species of the genus (or subgenus) *Notoedres*, *minor* and *alepis*, of which the first presents two varieties.

N. minor Fürstenberg, 1861 (Syn. *S. cati* Hering 1838, *catorum* Küchenmeister 1855, *cati* and *cuniculi* Gerlach 1857, *Sarcopte notoèdre* Delafond and Bourguignon 1862, *S. notoedres* Mégnin 1876, *felis* Gerlach 1877). The scales are represented by concentric undulating lines round the dorsal anus. There are four slender spines on either side of the notothorax and six stronger

but still slender spines on either side of the anus. Ambulacrum with peduncle relatively short and terminal sucker large (Railliet). Two hairs longer than the rostrum on the epistome; a single pair of short anal bristles (Neumann).

1. Var. *cati*.

♀ 245–280 μ \times 165–175 μ .

♂ 145–150 μ \times 120–125 μ .

The cat scabies mite was seen by Gohier in 1813 but it was Hering (1838) who first attempted to describe it. He apparently found the male, which he considered the smallest Arachnid known. Fürstenberg fused *cati* and *cuniculi*, but Gerlach thought them distinct, and Mégnin and Railliet take the same view, though the differences are practically only physiological.

The scabies begins on the head and ears of the cat, but later invades the whole body. Though easily cured in its early stages it causes death, if untreated, in 4–6 months. A great many cases prove that it is easily communicable to man, but soon dies out spontaneously. Gerlach experimented on pupils at Berlin and on himself, but the disease always died out in from 10 to 20 days. All the clinical cases of cat scabies on man have been traced to contact with pet cats in a mangy condition.

Horses have acquired it from mangy cats reposing on their backs, and the same is said of the ox, though this is questioned. Gerlach attempted, unsuccessfully, to transmit it to the pig. Delafond and Bourguignon gave it to the dog—puppies dying of the disease. Mégnin places here the sarcopt found by Colin on the Coati, but Railliet thinks it was *N. alepis*.

2. Var. *cuniculi* (*S. cuniculi* Gerlach, 1857). This must be the “variété gliricole” of Mégnin (1893, p. 145).

♀ 215–235 μ \times 165–175 μ .

♂ 142–155 μ \times 116–125 μ .

Beyond the trifling difference of measurements there seems to be no visible distinction between *cati* and *cuniculi*, but their behaviour is not the same. It is severe on the rabbit, beginning at the muzzle and then invading the whole body, frequently fatal, because the animal is unable to feed. According to Mégnin (p. 145) it also affects the rat. Railliet found it only very slightly contagious. He could not transmit it to the rabbit by depositing crusts from a mangy rabbit on the shaved skin of a healthy subject, nor by cohabitation for 11 days. He did not succeed in communicating it to the dog, the cat or the rat.

N. alepis Railliet and Lucet, 1893.

Striations regular and concentric; no dorsal scales; four very slender spines on either side of the notothorax, and six on either side of the anus, which is more posterior than in *N. minor* (Text-fig. 8).

♀ 300–450 μ \times 230–400 μ .

♂ 170–180 μ \times 130–140 μ .

Legros observed this parasite on rats at the Jardin des Plantes in 1865, and Railliet and Lucet (1893, p. 404) found it again on "white mice," the black rat and the water vole. The disease is localised on the ears and the genitals, and apparently is never serious.

Railliet attributes Colin's Coati sarcopt to this species.

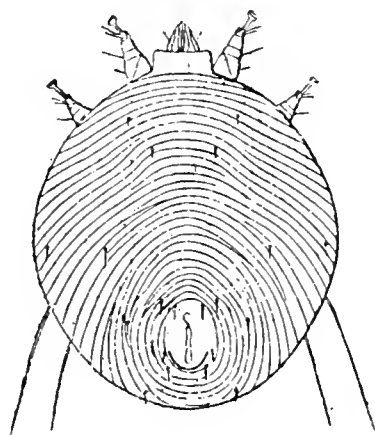
CNEMIDOCOPTES Fürstenberg 1870.

The *Tierreich* definition is:

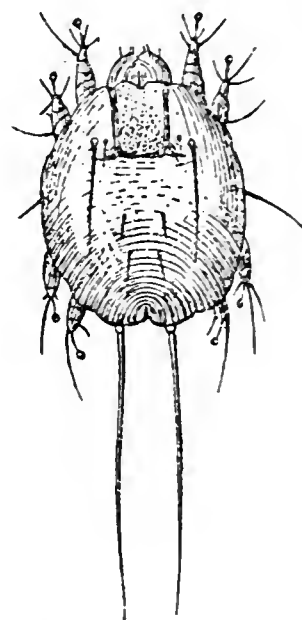
Male without anal cylinders; ♀ without copulation tubes; the mature ♀ without ambulacra; ♂ with long unjointed ambulacra on all legs; anus terminal.

*Oviparous*¹ (?) *parasites on birds.*

It is not proposed to deal in detail with this genus. It is entirely parasitic on birds, and its investigation would entail a quite separate branch of research.



Text-figure 8. *Notoedres alepis*, ♀ × 75
(after Railliet).



Text-figure 9. *Cnemidocoptes mutans*, ♂ × 100
(after Neumann).

Cnemidocoptes agrees with *Sarcoptes* and *Notoedres* in the nature of its mouth-parts and in its burrowing habits, and the type species *mutans* Robin, 1859 has long been known as causing scales and crusts on the domestic and game birds, and also on small birds in aviaries. Other species, however, have habits less akin to the usual sarcoptic method of life, and burrow into the feather-bulbs, causing deplumation. It will be sufficient to allude very briefly to the different forms which have been described. The general view seems to be that there are two species, *mutans* and *laevis*, with several varieties of the latter.

C. mutans Robin and Lanquetin, 1859.

Railliet (1895, p. 663) describes it thus:

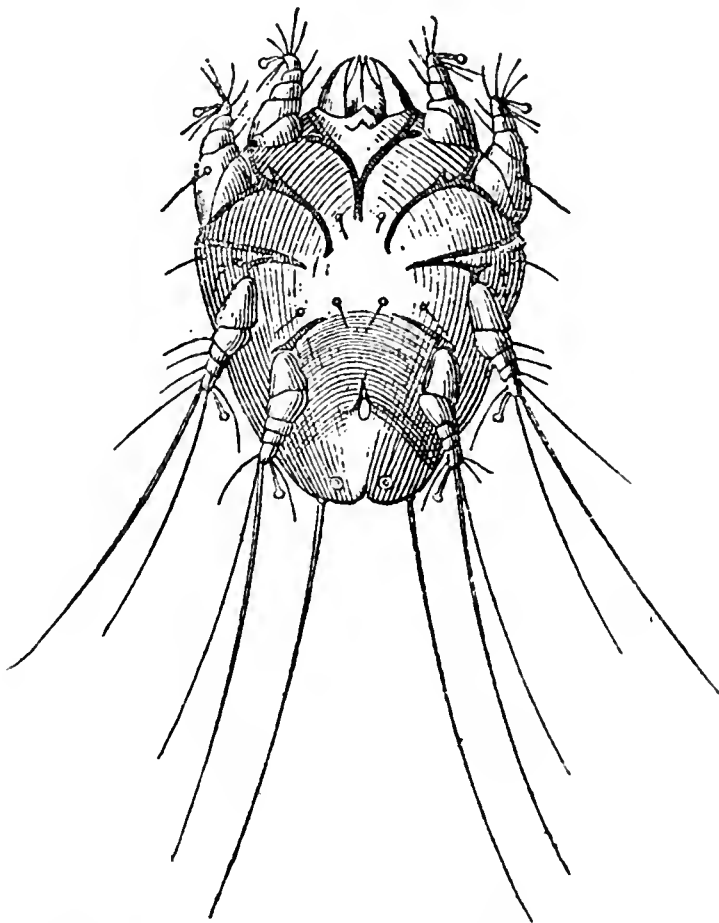
Rostrum broad, half concealed by epistome; ♂ 190–200 μ × 120–130 μ , body oval; no cheeks; conical legs, all with ambulacra; genital armature between legs 4.

¹ Fürstenberg calls *C. mutans* viviparous, and Railliet figures *gallinae* ♀ with obvious larvae inside.

♀ 408–440 μ \times 330–380 μ ; very short oval; abdomen (at first) as large as cephalothorax; rostrum with very broad carinate cheeks, filling the interval between legs 1 and head; dorsum mammillated; transverse tocostome just behind epimeres of legs 2; legs reduced to short conical stumps without ambulacra.

This sarcopt has been known for a long time on domestic fowls and other Gallinaceae and on game birds. It has also been observed to infest small birds in aviaries. It burrows under the scales of the legs and causes large spongy crusts which are full of the mites.

Note the absence of “cheeks” in the ♂, and the presence of very broad well-developed cheeks in the ♀.



Text-figure 10. *Cnemidocoptes laevis* var. *gallinae*, ♂ \times 200 (after Railliet).

C. laevis Railliet, 1885.

Railliet (1895, p. 664) describes it thus:

Rostrum broad, half covered by the epistome; ♂ oval, without cheeks; genital armature between legs 4; two copulatory suckers one on either side of the anus.

♀ rounded; abdomen a little larger than cephalothorax; cheeks as in *mutans*; dorsum regularly striate, without mammillae; tocostome faint; legs as in *mutans*.

This species causes “body” or “depluming” scabies, and was first found on a carrier pigeon from Brussels. Railliet says it appears in poultry yards in consequence of the introduction of infested fowls and quickly invades the whole run. It usually begins on the rump and spreads to thighs, back and belly, and the feathers fall off. The fowls do not usually suffer much in health,

but deteriorate in flesh and in egg-laying. Cocks suffer most, and sometimes die in a cachectic state. The disease is most prevalent in spring and summer.

The varieties of *laevis* which have been founded by various writers are:

Fossor Ehlers, 1873, on the passerine bird *Munia maja*, and *glaberrimus* and *philomelae* described by Sicher, 1893, from *Dendrocopus medius* and *Luscinia philomela* respectively.

Railliet's type species is sometimes known as *C. laevis* var. *gallinae*. Ehlers (1873, "Die Krätzmilben der Vögel," *Zeitschr. f. wiss. Zool.*, pp. 228-253, Pls. XII and XIII) gives beautiful figures of *fossor* (under the genus *Dermatoryctes*). Its chief characteristic is the possession of four claws on the female tarsus. The epimeres of legs 1 are free.

Sicher (1893, pp. 134 *et seq.*) describes *glaberrimus* and *philomelae*:

Glaberrimus has the epimeres of legs 1 free, and no posterior hairs; in *philomelae* the epimeres of legs 1 meet at an obtuse angle, and there are six hairs of about equal length at the posterior end of the body.

The males are not known.

CONCLUSIONS.

We have little doubt that future investigators will regard *Sarcoptes*, *Notoedres* and *Cnemidocoptes* as generically distinct. *Cnemidocoptes*, always parasitic on birds, and comprising members of different habits—scab forming or depluming—would seem to form a compact group for a separate research. *Notoedres*, attacking small mammals, but often communicated by them to the larger mammalia and to man, cannot well be dissociated from *Sarcoptes* in any study of the acarine causes of scabies, but the foregoing résumé of our present knowledge of the subject makes it abundantly clear that the urgent and immediate need is to clear up the confusion which exists with regard to the last-named genus. Whether different forms are to be regarded as species, varieties or races is, after all, a minor matter, and one as to which differences of opinion may well continue to exist after the most thorough investigation. What is important is that one form should be so completely studied that differences of structure in forms thought to be distinct from it may be clearly recognised, and when this has once been accomplished a foundation will at least have been laid for a comparison between the *Sarcoptes* of the different animals. The human *Sarcoptes* would probably be selected for this purpose. If so, care should be taken that the patients who supply the material should not be men occupied in the tending of domestic animals, and especially of horses, lest there be any suspicion of contagion from such sources. There would be some advantages in selecting instead the form known as *equi*, which is easily obtainable, and in which the salient characteristics would seem to be more strongly marked.

Any structural peculiarity which has been noted in *any* so-called species or variety should be specially looked for in the type selected for exhaustive study, Mégnin's experience being always borne in mind. He made a new

species based on characters which, when deliberately sought for, were found to be present in all the forms he was able to examine.

Chaetotaxy is always important in the Acarina, and should be studied with especial care. The bristles and hairs are difficult to see in a transparent medium, and particularly so in Canada balsam which has nearly the same refractive index. It is absolutely necessary to examine them in some medium which shall render them more visible. It will be noticed that in the Plate the hairs of Munro's figures of *S. scabiei* are much longer than those depicted by any other investigator, and this is entirely due to his viewing them in a gum-arabic medium. It is possible that picric acid or some other reagent might stain them faintly, and there is a third method—of viewing the specimen in a coloured field of some colloid pigment, when unsuspected length of bristles is often betrayed by the length of the light tracts traced by them on the coloured background. The particular distribution and the comparative length of these bristles would, if constant, be good specific characters.

The observations of Fürstenberg and others on the dorsal scales, the notothoracic cones and the notogastral spines should be repeated and extended. If, for example, Fürstenberg is accurate in his delineation of the dorsal scales of the sarcopt of man and of the sarcopt of the dog there can be no doubt that these two forms are essentially distinct.

The leg armature and chitinous frame-work, and the epimeres should once more be subjected to a very close scrutiny. Fürstenberg and others have delineated the skeletal structures of the legs under a high magnification, and with considerable detail of structure. Nevertheless it will be noted that not a single one of the best accredited figures of the human sarcopt shows the presence of a trochantal claw on the anterior legs. Canestrini figures it very clearly in the case of two other "species" (*e.g.* see Canestrini, VI. Pl. 60) and Mégnin detected it in a horse sarcopt which he judged to be a new species—largely on account of its presence. When, however, he re-examined the human *Sarcoptes* for the express purpose of finding it he was at once successful. In fact he was driven to the conclusion that it was a constant feature of all the forms of *Sarcoptes*.

If there are real specific differences between the various forms of *Sarcoptes*, one would expect them to be indicated in their most fixed, and strongly chitinised structures, the epimeres. Slight differences are alleged to exist in these epimeres, especially those of the second pair of legs, which are sometimes nearly straight, at others sharply bent outwards at their free extremities. Their extremities, also, may present useful differences, and be knobbed or forked as the case may be. All these details will naturally engage the attention of anyone undertaking a revision of the subject.

In the diagnoses of the various forms mention is generally made of certain clear areas (*clairières*, Blösse), on the dorsum. It appears to me that the terms are used in a double sense, sometimes indicating simply a region where the striations are absent (as on the notogaster of the female), but more often

used to denote a definite area with a granular or rugose surface and sometimes more distinctly chitinised than the rest of the integument. These are what Munro calls "rugose areas" and what he very diagrammatically depicts as dark patches, one on the notothorax of both ♂ and ♀, and two on the notogaster of the ♂. Mégnin says that in *equi* the notothoracic rugose area (plastron) is of a faint yellow colour. In the human sarcopt it seems to be very inconspicuous, and it does not appear at all in many of the best accredited figures of this form. Gerlach shows it as a bare, somewhat rectangular light patch. It is present in all the varieties, and may perhaps be regarded as a sort of rudimentary scutum, such as is found in many arachnids. Its shape and dimensions are considered to be of taxonomic importance, and indeed, if it be of the nature of a scutum, it is just one of those structures in which forms which are really distinct might be expected to exhibit differences.

In the Acarina for the most part the males of the various species are more distinctive than the females. So far, in the genus *Sarcoptes*, only very slight differences between the males of the various forms have been noted, the most important being the relation of the epimeres of the posterior legs to the epiandrium. The smaller size, and the relatively small number of the males, may have militated against their receiving due attention, but if they are all as similar as the various observers have apparently found them this would certainly argue a very close affinity between the different forms of *Sarcoptes*.

When once the chaetotaxy and armature of a single form have been definitely established, *Sarcoptes* taken from characteristic cases of scabies in other animals may be compared with it as occasion arises. All possible care must again be taken that the case shall be one of the ordinary scabies of the animal, and not a temporary infection from an animal of a different group.

Much reliance has been placed upon mere size. When the very wide range given in the measurements of the adult female and male of all the so-called species is considered, it is not easy to believe in the great importance of this character. It would at most indicate a distinction of race in the absence of more significant differences of structure. Some writers have arranged the varieties they recognise in order of size. Dubreuilh and Beille, for instance (p. 12), give as a descending series *suis*, *equi*, *lupi*, *caprae*, *cameli* (our *dromedarii*), *ovis* and *hominis*, the first-named attaining 500 μ in length and the last 300 μ . It is admitted that, notwithstanding considerable inequality in the size of individuals, there may still be a noticeable difference in average size in the forms found on different hosts. Care should at least be taken that the average size attributed to any form be based on the measurement of a sufficient number of specimens. Where transference from one animal to another is possible it would be a very interesting observation to note whether it is accompanied by any change in average size.

The proposed research will no doubt include experiments in the artificial transference of scabies from one animal to another, and the views—and the warnings—of Delafond and Bourguignon in this connection will be especially

interesting, whatever the final verdict may be concerning them. They regarded the domestic animals as the constant reservoir from which man renewed his supply of *Sarcoptes*, which would otherwise have been exterminated by its very easy treatment. They believed that for scabies to flourish in any of the lower mammalia a poor condition of health was necessary, and that man, and perhaps monkeys, are the only exceptions to this rule. And they warn us repeatedly against drawing any conclusions from failure to communicate scabies to another animal by the transference of a limited number of possibly injured *Sarcoptes* from a given host.

In all the cases in which severe scabies has been successfully and quickly communicated to a different animal the first host has been in an advanced stage of the disease, exhibiting crusts which are swarming with the mites in all stages. Contagion from animals only slightly affected by scabies is always slow and uncertain. Human scabies (except in the form of Norwegian itch) is almost always relatively slight, presenting no crusts, and it is notorious that doctors never contract itch from their patients, and that even when a man has become infected from occupying the bed of a scabietic person weeks may elapse before he becomes aware of the fact. Again it very often happens in these experiments that the transference is successful for a time but that the disease presently dies out. The possible explanation that the recipient host is in a condition of health unfavourable to the establishment of the disease must not be lost sight of. It was not till the bear and the hyaena in the Ménagerie Pianet had greatly lost condition from other causes, that they contracted scabies from the mangy lions, and many parallel cases have been recorded.

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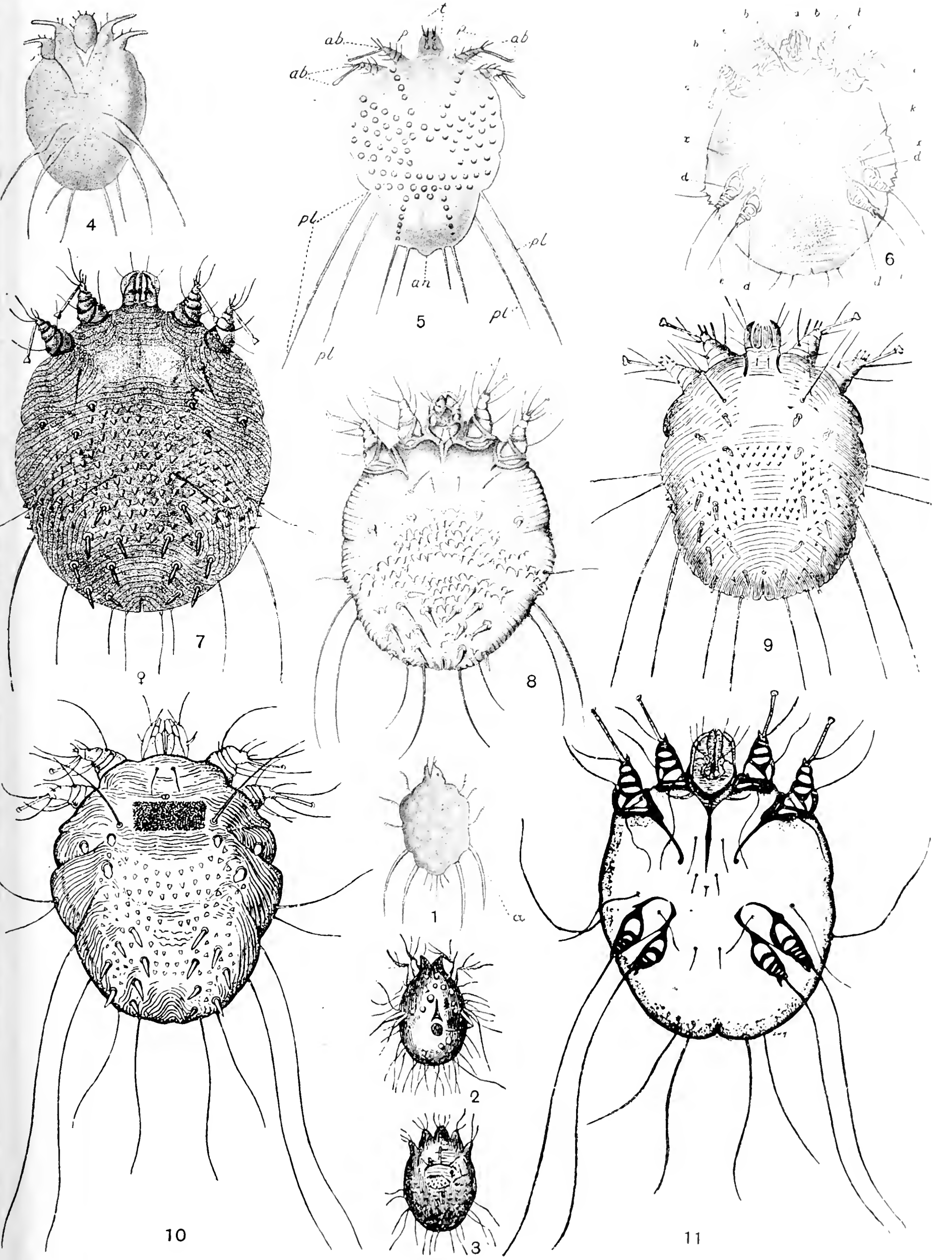
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DESCRIPTION OF PLATE XV.

Figures of *S. scabiei* de Geer given by different investigators from 1687 to 1919.

- Fig. 1. Bonomo's (? Cestoni's) figure, regarded as the classical representation of the mite from 1687 to 1750. (From Raspail, 1838, Pl. XV, fig. 14.)
- Figs. 2 and 3. Linnaeus' figures of *Acarus exulcerans* and *A. scabiei* according to Wichmann. (From Raspail, 1834-46, p. 111.)
- Fig. 4. De Geer's figure, 1778. (From Raspail, 1838, Pl. XV, fig. 11.)
- Fig. 5. The mite as seen by Raspail. (Raspail, 1838, Pl. XV, fig. 1.)
- Fig. 6. Delaford and Bourguignon's figure (*Traité pratique*, Pl. I, fig. 1), practically a reproduction of Bourguignon's figure of 1852.
- Fig. 7. Gerlach's figure, 1857. (Gerlach, Pl. I, fig. 1.)
- Fig. 8. The mite according to Fürstenberg, 1861. (Fürstenberg, Pl. I, fig. 7.)
- Fig. 9. Blanchard's figure, 1889. (From Brumpt, fig. 322.)
- Figs. 10 and 11. Dorsal and ventral aspects of *S. scabiei* according to Munro, 1919. (Munro, figs. 1 and 2.)



A CONTRIBUTION TO OUR KNOWLEDGE OF THE
TAPEWORMS OF POULTRY.

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(With Plate XVI and Plate XVII, Figs. 8-11, and 1 Text-figure.)

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THE following paper is an account of the results obtained by the study of Cestodes sent to this Department for identification. For the most part, the tapeworms were species with a wide distribution and with many previous records of their occurrence. Among them were several whose anatomy, well-known in many respects, had been inadequately described with regard to characters useful, if not essential, for identification.

The present paper, with the exception of that portion concerning the new species *Cotugnia fastigata*, is an attempt to deal with such characters and so complete already existing descriptions.

I wish here to express my indebtedness to Prof. Nuttall, F.R.S., for specimens of *Hymenolepis columbae* (Zed.), *H. coronula* (Duj.), *H. gracilis* (Zed.), and *Cotugnia fastigata* n. sp., collected by Dr H. H. Marshall at Rangoon, and to Dr C. L. Boulenger for those of *Cotugnia digonophora* (Pasq.), from Mesopotamia.

COTUGNIA DIGONOPHORA (PASQUALE, 1890).

The genus *Cotugnia* was created by Diamare (1893) for those avian Cestodes which have a double set of male and female reproductive organs in each proglottis, and a rostellum armed with T-shaped hooks. The type species is *C. digonophora* (Pasq.), a form described under the name of *Taenia digonophora* by Pasquale in 1890 from chickens in Abyssinia. Since that date,

with the exception of a paper by Khitrow (1900) inaccessible to me, the species has not been recorded. Pasquale's description is incomplete in many respects so that, although through the work of Fuhrmann (1909) on the species *C. crassa* Fuhr., *C. collini* Fuhr. and *C. polycantha* Fuhr., the genus is well defined, the type species has remained practically undescribed. Three complete tapeworms from chickens in Mesopotamia were handed to me for identification. These I believe to be specimens of *C. digonophora* (Pasq.), and take the opportunity to compile as full an account as possible of the anatomy of this type species.

The sizes of the strobilae are 107 mm. \times 4 mm., 80 mm. \times 4 mm., 26 mm. \times 2.5 mm. and 22 mm. \times 2.5 mm. The scolex (Pl. XVII, fig. 9) is 0.66 mm. long \times 1.07 mm. broad, with four unarmed suckers 0.36 mm. long \times 0.25 mm. wide, arranged two dorsally and two ventrally. The rostellum is 0.16 mm. in diameter, armed with a double crown of hooks 0.0122 mm. long, of the usual *Davainea* shape, the hooks on each row being of the same size and alternating with each other, with but little difference in their anterior level. The rostellum itself is a simple muscular structure sunk between four large lobes which completely hide it. All proglottides are broader than long.

These measurements do not agree with those quoted by Stiles (1896, p. 30) "Strobila 40 mm. to 80 mm. by 8 mm. broad and in contracted condition about 1 mm. thick; head 1.4 mm. by 1.12 mm., rostellum with a crowded crown (in a single row) of very small hooks 8.35μ long; base of rostellum 0.22 mm. by 0.15 mm., suckers globular, prominent, 0.35 mm. in diameter. Neck short. Anterior segments broader than long, posterior segments longer than broad. Genital pores double in about the middle of the lateral margins; two ovaries in each segment; eggs evidently arranged in egg sacs."

These differences in the measurements and the shape of the proglottides, characters indefinite and liable to variation, are probably the result of individual variation in the tapeworms examined and can therefore be safely neglected.

The longitudinal musculature (Pl. XVII, fig. 11) is in two layers. The innermost (*l.m.*) consists of numerous bundles of loosely packed fibres, approximately 20 fibres to a bundle. Internally it is bounded by a single (*t.m.*) and externally by a double (*t'.m'.*) layer of transverse muscles, the two layers of the latter separated by a narrow stratum of parenchyma. Numerous dorso-ventral fibres cross these two last layers and pass between the inner longitudinal muscle bundles. Externally to the transverse muscles mentioned is a widely diffused layer of longitudinal muscles (*l'.m'.*). Internally these form very compact bundles of very few fibres, approximately 10 per bundle, but diminish gradually to isolated fibres which do not extend as far as the cuticle. Both these two longitudinal muscle layers extend posteriorly into segments containing mature eggs, but only the inner extends anteriorly into the scolex. There it is much stronger, the bundles being more numerous and more compact, and forming an almost continuous layer which ceases at the posterior half of the suckers.

The excretory system consists of the usual four longitudinal vessels, two dorsal and two ventral. The former are exceedingly minute and are external to the two latter. At the posterior limit of each proglottis, the two ventral vessels communicate by a large transverse commissure. No such commissure could be found between the two dorsal, probably on account of their small size. In segments filled with mature eggs, both dorsal and ventral vessels disappear. In the anterior segments they are of equal size. In the scolex they run between the suckers in a wavy course, giving off numerous anastomosing branches and opening into a circular commissure, immediately posterior to the rostellum.

There are two complete sets of genital organs in each proglottis (Pl. XVII, fig. 10). The genital pore is half way along each lateral margin or else slightly anterior. A very shallow genital cloaca is present. The genital canals run dorsal to both excretory vessels and to the nerve.

The cirrus-pouch is long and slender and devoid of special retractor muscles. It only extends as far as the nerve, not past it as Fuhrmann (1909, p. 120) states, "Der Cirrusbeutel ist langgestreckt (0.3 mm.) und geht über den Längsnerv bis zu dem nach aussen vom ventralen Exkretionsstamm gelegenendorsalen Exkretionsgefäss," and is straight. The cirrus is armed with fine spines and has a small terminal enlargement. It runs in a straight line half-way along the cirrus-pouch and then merges into the vas deferens; after this point the cirrus-pouch is less muscular. The vas deferens is twice the diameter of the cirrus and is uncoiled while still within the pouch. On emerging, it coils into a loose ball and then, running posteriorly, breaks up into the vasa efferentia. There is no vesicula seminalis, the coils of the vas deferens functioning instead. The testes (*t.*) form a broad band across the proglottis, are approximately 100 in number and are posterior to the female glands. None are to be found in the anterior half of the segment. They surround dorsally and laterally, but not ventrally, the longitudinal excretory canal and extend as far as the longitudinal nerve. Dorso-ventrally they form an indistinct double layer in the centre of the proglottis, being three deep at the extremities and only one deep in the centre.

The vagina (*v.*) opens posteriorly to the cirrus and runs slightly posteriorly to open into a spindle-shaped receptaculum seminis (*r.s.*) situated just internally to the excretory vessels. The ducts from the receptaculum seminis take their usual course. The ovary (*ov.*) is crescent shaped with the concavity directed posteriorly and internally, and with the convex side deeply lobed. The yolk-gland (*y.g.*) is a shapeless irregularly lobed gland lying posteriorly and internally to the ovary. Both ovary and yolk-gland lie immediately internally to the longitudinal excretory vessels with the ovary half-way between the posterior and anterior borders of the segment. The shell-gland is very compact, non-lobed, and lies on the dorsal surface between the ovary and yolk-gland. A definite uterus is not developed. The oviduct, after passing through the shell-gland, persists for an exceedingly short distance

as a rather wide indistinct parenchymatous tube filled with eggs and ends blindly in the parenchyma. In a proglottis at this stage of development the anterior half is already full of fertilised eggs; in the stage immediately preceding, the uterine tube has more definite walls but still ends blindly, while but few eggs are visible in the proglottis. In proglottides ready for detachment, the eggs fill the entire segment, extending laterally to the excretory vessels almost to the margin of the segment. Adjacent proglottides are separated only by a thin plate of parenchyma. The egg is ellipsoidal, measuring 0.63 mm. \times 0.583 mm. and the onchosphere 0.288 mm. \times 0.249 mm.

COTUGNIA BROTOGERYS (MEGGITT, 1915).

This species from a parakeet, *Brotogerys tirica*, has already been described in detail (Meggitt, 1915). Revision of the material has recently shown that this description is inaccurate in one respect. It has been stated (p. 54) that the parenchymatous capsules each contain several ova. This is not correct. In young segments the eggs lie singly in separate capsules. As the proglottis matures the number of eggs increases and the capsules become packed so closely together that in many cases the walls separating them disappear. The capsules thus appear each to contain several ova.

The testes surround the longitudinal excretory vessel except on the dorsal surface, a condition exactly opposite to that in *C. digonophora*. Antero-posteriorly, the band of testes consists of five rows; dorso-ventrally they lie in the centre of the proglottis.

COTUGNIA FASTIGATA N. SP.

Amongst a large number of *H. coronula* (Duj.) from domestic ducks, Rangoon, were found several complete specimens of a *Cotugnia*. Upon examination these proved to represent a new species for which I propose the name *Cotugnia fastigata* n. sp. and which may be identified by the key attached to the following description.

The length is 30 mm., the breadth 6 mm., and all the proglottides are broader than long. In shape the strobila (Pl. XVI, fig. 4) is triangular, widest at the base and narrowing gradually towards the head. The scolex is 0.5–0.6 mm. diameter, and is provided with four unarmed suckers and an armed rostellum. The latter is 0.29 mm. diameter, and bears approximately 200 hooks, 0.02 mm. long, arranged in a double row, the hooks of the two rows alternating. It proved impossible to ascertain the exact shape of the hooks owing to the difficulty of isolating them; one figured in Text-fig. 1 is from a section and has obviously been cut at the place marked *x*. The base of each hook (Pl. XVII, fig. 8) has a fine sheath of muscle (*m.*) surrounding it. Posteriorly this merges into a fine muscle strand 0.5 μ diameter which, uniting with adjacent muscle strands, ultimately joins the body longitudinal muscles. Externally to the hooks and surrounding the rostellum is a thin sheath of transverse muscles.

The scolex (Pl. XVI, fig. 7) is attached to several villi which, free at first, in course of time become fused together into a solid mass at their distal ends but with the proximal ends still showing traces of their original formation. To this thickened lump the scolex fixes itself by the rostellor hooks. The centre of the rostellum, instead of being applied closely to the mass of villi, retracts, so as to leave between the scolex and the intestine a conical cavity, often partially filled with mucous, round the circumference of whose base the rostellum is still firmly fixed by its hooks. The suckers appear to play a temporary part in the fixation of the worm to the intestine; immediately on death they relax their hold while the rostellum generally remains firmly imbedded. The scolex may hang freely in the lumen of the intestine or may be sunk among the villi, but it never penetrates the submucosa.



Text-fig. 1. *Cotugnia fastigata* n. sp., Hook from Rostellum.

The musculature (Pl. XVI, fig. 6) consists of three layers of longitudinal muscles each bounded internally by a thin layer of transverse ones. The innermost longitudinal musculature (*l.m.*) consists of a number of fibres aggregated together into large irregular bundles; the bundles of the middle layer (*l'.m'.*) are smaller, more compact, composed of fewer fibres, and arranged more regularly, while externally to them isolated fibres (*l''.m''.*) extend up to the cuticula.

The dorsal longitudinal excretory vessels are absent from proglottides containing genital organs; the ventral persist to the posterior end of the strobilus, and communicate by the usual transverse commissure at the posterior border of each proglottis.

The genital pore (Pl. XVI, fig. 5) is situated at the limit of the anterior quarter of the proglottis margin, and into it open anteriorly and posteriorly the cirrus-sac and vagina respectively.

The testes (*t.*) extend as a narrow band along the posterior border of the proglottis. Antero-posteriorly, this band has a uniform width, consisting of two to three rows of testes; dorso-ventrally, it is thickest laterally where

there are three layers of testes extending from the dorsal to the ventral surface and thinnest in the centre where there is only a single layer on the dorsal surface. Laterally the testes surround the ventral excretory canal and extend as far as the nerve. There is also a small group of testes situated between the ovary and the excretory canal. The anterior portion of the proglottis is entirely free from them. The cirrus-sac (c.s.) is small and narrow but long in proportion to its width, and extends as far as the lateral nerve. Between it and the testes, the vas deferens forms a closely packed bundle of coils surrounded by numerous gland cells. Inside the sac, the vas deferens coils once or twice and then opens into the short straight cirrus.

The vagina runs in a straight course laterally to open into a small spindle-shaped receptaculum seminis (r.s.). The ovary (ov.) is deeply divided into several blunt lobes closely packed together. It lies posterior and ventral to the receptaculum seminis and close to the excretory canal. Posterior and slightly aporal to it is the compact yolk-gland (y.g.) surrounded laterally by the testes.

The uterus (u.) is present as a narrow branched tube anterior to the ovary, but soon disappears, the mature eggs lying singly in capsules in the parenchyma.

KEY TO SPECIES OF COTUGNIA.

1. Rostellum smaller than suckers	<i>C. margareta</i> Beddard
Rostellum larger than suckers	(2)
2. Testes 6-7, anterior to female glands	<i>C. browni</i> Smith
Testes numerous, lateral or posterior to female glands	(3)
3. Testes clearly divided into two lateral groups	(4)
Testes in one broad posterior band	(5)
4. Testes in one layer transversely	<i>C. collini</i> Fuhr.
Testes in several layers transversely	<i>C. polycantha</i> Fuhr.
5. Cirrus-sac extending beyond ventral longitudinal excretory vessel	<i>C. fuhrmanni</i> Bacz.
Cirrus-sac not reaching ventral longitudinal excretory vessel	(6)
6. Yolk-gland lateral to ovary	<i>C. crassa</i> Fuhr.
Yolk-gland posterior to ovary	(7)
7. Testes 2-3 rows antero-posteriorly	<i>C. fastigata</i> n. sp.
Testes 5 rows antero-posteriorly	(8)
8. Testes dorsal and lateral to longitudinal excretory vessels	<i>C. digonophora</i> (Pasq.)
Testes ventral and lateral to longitudinal excretory vessels	<i>C. brotogerys</i> Meggitt

(*C. inaequalis* Fuhrmann 1909 has not been described sufficiently to allow of its inclusion in this table.)

HYMENOLEPIS COLUMBAE (ZEDER, 1800).

The specimens of this species had been collected from pigeons, Rangoon, and consisted of portions of strobila in various stages of development. No scoleces were found. According to Ransom (1909, p. 97) this species is identical with the *H. sphenoccephala* (Rud.) described by Fuhrmann (1906, p. 449), whose description, with the exception of the points mentioned below, was fully corroborated by my preparations.

The genital pore is much more anterior than is figured by him, lying in the anterior quarter of the proglottis; the genital cloaca is also much shallower, being only a small depression. The vagina consists of two separate parts. The first, muscular and with an extremely wide lumen, runs dorsally from the genital cloaca as far as the inner limit of the sacculus accessorius, there to open into a more muscular duct with no apparent lumen. This latter portion is spirally coiled, and runs ventrally and anteriorly to open into the receptaculum seminis. The difference between the two parts of the vagina is very marked. Of the two aporal testes, Fuhrmann (1906 *a*, p. 450) states "der vordere (liegt) aber etwas ausserhalb des hinteren antiporale Hodens." Preparations and sections showed this clearly. Though the two testes often appeared to lie one directly anterior to the other, yet in every case a projecting tongue from the anterior one lay external to the posterior, and a distinction between internal and external could be made.

HYMENOLEPIS CORONULA (DUJARDIN, 1845).

This cestode of ducks has been described by many authors, in particular by Wolffhügel (1900, p. 165), but although most of its anatomy is well-known the following points have escaped attention. The specimens I received were taken from domestic ducks in Rangoon.

The three testes (Pl. XVI, fig. 1, *t.*) are arranged as Fuhrmann (1906 *a*, p. 733) states, one poral and two aporal, the edges of the poral and the inner aporal testes touching in young segments but being gradually separated by the growth of the female glands between them. The arrangement of the two aporal testes varies according to the state of contraction of the segment; in proglottides extremely contracted, as in the one figured, the three testes are in the same straight line; in proglottides extended, one aporal testis lies anterior to and internal to the other. In all cases though, the outer testis has a small projection lying posteriorly to the inner, so that even when in the same straight line a distinction into anterior testes and posterior testis can be made. Wolffhügel (p. 171) states, "Die drei Hoden erreichen bei *Dicranotaenia coronula* in höchster Reife eine solche Grösse, dass sie bis an die dorsale und ventrale Längsmuskulatur stossen." This I have not found to be the case, the three testes lying well within the musculature. The cirrus-sac (*c.s.*) extends as far as, or just passes, the ventral longitudinal excretory vessel, and is two-thirds filled by the internal vesicula seminalis (*v'.s'*), rather a larger proportion than figured by Wolffhügel (Pl. VII, fig. 103). The cirrus extending from this vesicula seminalis to the genital pore is straight and the small sacculus accessorius present is a straight tube of uniform diameter with no terminal enlargement; both these observations do not agree with the figure above mentioned. The external vesicula seminalis (*v.s.*) is small, and spindle shaped, and lies dorsal and lateral to the receptaculum seminis.

The female glands (Pl. XVI, fig. 2) are posterior and ventral, and occupy one-third of the proglottis breadth. The bluntly lobed yolk-gland (*y.g.*) lies

on the posterior and ventral surface of the segment and is surrounded on all sides except the posterior by the narrow unlobed ovary (*ov.*).

When fully developed, the receptaculum seminis (*r.s.*) extends from the ventral longitudinal excretory vessel to a little over half-way across the segment. It is slightly funnel-shaped, the inner end being rather larger than the outer. The uterus (*u.*) in its early stages is a long narrow sac twisted upon itself so that its cavity appears to be divided by septa. Later it increases in size and occupies the whole of the proglottis, extending past the excretory vessel as far as the cuticula. At this stage the proglottis is a mere egg-sac, but with no communication with adjacent segments. As was the case with other observers, no mature eggs could be found.

HYMENOLEPIS GRACILIS (ZEDER, 1803).

The specimens of this species were obtained from the intestine of domestic ducks, Rangoon, and sections confirm Wolffhügel's account. The following additional points were noticed. The genital pore (Pl. XVI, fig. 3, *g.p.*) is extremely anterior, in the anterior quarter of the proglottis margin, and is often covered by the overlapping of the preceding segment. The cirrus-sac (*c.s.*) does not, as Lühe (1910, p. 61) states, barely reach the centre line of the proglottis, but extends well past it, nearly to the aporal longitudinal excretory vessel. The testes (*t.*) are arranged as Fuhrmann (1906 *b*, p. 733) states, one poral and two aporal; of the latter the one is always external and anterior to the other. As stated by other investigators no transverse commissures could be traced between the longitudinal excretory vessels of opposite sides. A large number of minute branches are given off during their course, particularly in the neighbourhood of the posterior margin of the proglottis and as these latter branches penetrate some distance into the segment and could often be traced half-way across it, it is a reasonable supposition that through them the opposite excretory vessels communicate.

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For description of Plates XVI and XVII see p. 313.

A NEW SPECIES OF CESTODE (*OOCHORISTICA* *ERINACEI*) FROM THE HEDGEHOG.

BY F. J. MEGGITT, M.Sc., Ph.D.,

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University of Birmingham.*)

(With Plate XVII, Figs. 12-13.)

A NUMBER of Cestodes collected from a hedgehog in Mesopotamia by Dr Boulenger were handed to me for identification. Examination showed them to belong to the genus *Oochoristica*. Five Cestodes [*Hymenolepis erinacei* (Gm.), *Hymenolepis steudeneri* v. Janicki, *Davainea parva* v. Janicki, *Taenia voluta* v. Linstow, and a *Bothriocephalus* larva v. Janicki] have up to the present been recorded from this host. From all these, the present species is distinguished by its possession of numerous testes, situated posteriorly to the female organs. I therefore propose to form a new species for its reception and suggest for it the name *Oochoristica erinacei*. The key attached to the following description will serve to distinguish this new species from others of the same genus. I wish here to express my indebtedness to Dr Boulenger for placing his material at my disposal.

The length of the largest specimen is 15 mm. and the width 1 mm. The scolex (Pl. XVII, fig. 12) is 0.33 mm. diameter, provided with four suckers 0.165 mm. long \times 0.12 mm. broad, arranged in pairs, the members of each pair having their margins touching and being widely separated from the opposite pair. Anteriorly each sucker has a small opening leading to a slight furrow on the apex of the scolex, the whole having a similar appearance to that figured by Cohn (1903, p. 61, Text-fig. 6) for *O. surinamensis*. There is neither rostellum nor hooks. A neck is absent, segmentation starting immediately posteriorly to the suckers.

As figured by Beddard for *O. marmosae* (1914, Text-fig. 149) the strobilus swells out into a collar immediately posteriorly to the scolex. The musculature consists of an inner layer of transverse muscles, externally a layer of comparatively strong longitudinal ones, and externally to those a very weak layer of scattered fibres, only present in the more anterior segments and disappearing in those with fully developed genital organs.

There are the usual four longitudinal canals, two ventral and two dorsal, communicating by a circular commissure at the posterior end of each proglottis. The two dorsal are small, and the two ventral relatively large, the commissures connecting the latter being often one-third the dorso-ventral diameter of the proglottis. Besides these four vessels there is an extensive irregular meshwork of ill-defined vessels and lacunae. Of the additional longitudinal vessels described by investigators and stated to be characteristic of the genus I have been unable to find any trace. The network referred to above is of far too vague a character to correspond in any way with a definite system such as is to be found in the other species, *e.g.* *O. tetragonocephala* (Bremser).

The genital pore (Pl. XVII, fig. 13) irregularly alternates and is situated near the anterior extremity of the proglottis. It leads into a well-developed genital cloaca into which the vagina and cirrus open in the usual manner. The genital ducts pass between the dorsal and ventral excretory vessels and dorsal to the nerve. The male organs are fully developed in proglottides slightly broader than long, the female in those longer than broad. The cirrus-sac (*c.s.*) opens into the genital cloaca dorsally to the vagina. Lying diagonally, it extends well past the ventral longitudinal vessel, in some cases half-way across the segment. The cirrus is unarmed, has a small terminal knob, and coils slightly in the cirrus-sac. The vas deferens inside the sac is also coiled. It leaves the cirrus-pouch at the inner extremity on the ventral side, and, coiling once or twice, proceeds half-way across the proglottis near the dorsal surface, immediately dorsal to the uterus but not passing between the lobes of the ovary. At the level of the anterior extremity of the vitelline gland it breaks up into a number of vasa efferentia. The testes (*t.*) are between 30–50 in number and lie posteriorly and laterally to the female glands, not extending anteriorly further than the ovary. They form one layer dorso-ventrally and do not extend laterally beyond the longitudinal excretory vessels.

The vagina (*v.*) opens at the posterior limit of the genital cloaca and runs transversely to half-way between the two ventral longitudinal vessels. After coiling once or twice it opens into the oviduct in the centre of the proglottis. A receptaculum seminis is not present. The bilobed ovary (*ov.*) is situated in the anterior third of the proglottis, the basal portion being dorsal and the two lobes reaching the ventral side. Between these two lobes lie the greater part of the courses of the vagina, uterus, and oviduct. The latter springs from the ventral surface of the common basal portion of the ovary and proceeds in a straight line posteriorly to meet the vagina. From this meeting place the oviduct curves first posteriorly, then anteriorly to the ventral surface of the proglottis, receiving at the posterior limit of its course the vitelline duct. On the ventral surface it runs anteriorly past the level of the vaginal pore, then bends at right angles to run across the segment to the dorsal surface where it opens into the uterus. The yolk-gland (*y.g.*) lies

posteriorly to the centre of the proglottis, half-way between dorsal and ventral surfaces. It is bilobed, the lobes being directed anteriorly. The vitelline duct arises from the common posterior basal portion and runs anteriorly, at first on the ventral surface, later crossing the segment to open in the centre into the oviduct.

At its first appearance the uterus is an amorphous sac situated anteriorly and, in a transverse section through that region, occupying the whole of the proglottis. As it develops it extends posteriorly and sends out numbers of branches which ramify in all directions. This process continues, the whole uterus resolving itself into a meshwork of tubes; these ultimately form capsules containing at first several, but later only a single egg.

KEY TO SPECIES OF OOCHORISTICA.

- | | | | | | |
|---|-----|-----|-----|-----|---------------------------------------|
| 1. Egg-capsules contain several eggs | ... | ... | ... | ... | <i>O. megastoma</i> (Dies.) |
| Egg-capsules contain only one egg | ... | ... | ... | ... | (2) |
| 2. Testes extend anteriorly to ovary | ... | ... | ... | ... | (3) |
| Testes posterior, or lateral and posterior to ovary | ... | ... | ... | ... | (4) |
| 3. Host, <i>Amphisbaena alba</i> | ... | ... | ... | ... | <i>O. amphisbaena</i> (Rud.) |
| Host, <i>Zonurus tropidosternum</i> | ... | ... | ... | ... | <i>O. zonuri</i> Bayliss |
| 4. Vagina opens anteriorly to cirrus | ... | ... | ... | ... | (5) |
| Vagina opens posteriorly to cirrus | ... | ... | ... | ... | (6) |
| 5. Genital ducts pass between longitudinal excretory vessels | ... | ... | ... | ... | <i>O. tetragonocephala</i> (Brem.) |
| Genital ducts pass dorsally to longitudinal excretory vessels | ... | ... | ... | ... | <i>O. didelphydis</i> (Rud.) |
| 6. Testes lateral and posterior to ovary | ... | ... | ... | ... | (7) |
| Testes posterior to ovary | ... | ... | ... | ... | (11) |
| 7. Rostellum absent | ... | ... | ... | ... | <i>O. cryptobothrium</i> (v. Linstow) |
| Rostellum present | ... | ... | ... | ... | (8) |
| 8. Testes 50 | ... | ... | ... | ... | <i>O. incisa</i> Marotel |
| Testes 100 or more | ... | ... | ... | ... | (9) |
| 9. Cirrus-sac not reaching outer longitudinal excretory vessel | ... | ... | ... | ... | <i>O. marmosae</i> Bedd. |
| Cirrus-sac reaching or passing outer longitudinal excretory vessel... | ... | ... | ... | ... | (10) |
| 10. Genital ducts pass between longitudinal excretory vessels | ... | ... | ... | ... | <i>O. rostellata</i> Zsch. |
| Genital ducts pass dorsally to longitudinal excretory vessels | ... | ... | ... | ... | <i>O. surinamensis</i> Cohn |
| 11. Eggs absent from centre of proglottis | ... | ... | ... | ... | <i>O. bivittata</i> v. Jan. |
| Eggs present in centre of proglottis... | ... | ... | ... | ... | (12) |
| 12. Testes 15-20 | ... | ... | ... | ... | <i>O. tuberculata</i> (Krabbe) |
| Testes 20-30 | ... | ... | ... | ... | <i>O. truncata</i> (Rud.) |
| Testes over 30 | ... | ... | ... | ... | (13) |
| 13. Receptaculum seminis present | ... | ... | ... | ... | (14) |
| Receptaculum seminis absent | ... | ... | ... | ... | (15) |
| 14. Testes 39-46 | ... | ... | ... | ... | <i>O. agamae</i> Bayliss |
| Testes 70-80 | ... | ... | ... | ... | <i>O. wagneri</i> v. Jan. |
| 15. Eight longitudinal excretory vessels | ... | ... | ... | ... | <i>O. sp</i> Beddard |
| Four | „ | „ | „ | ... | <i>O. erinacei</i> n. sp. |

(*Oochoristica pseudopodia* (Krabbe), *O. murina* (Rud.), and *O. sp?* von Janicki 1906, are not included in the above key, their anatomy not having been sufficiently investigated.)

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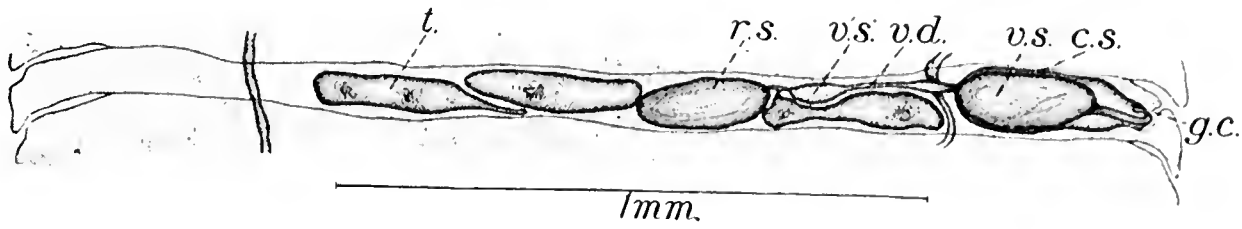


Fig. 1

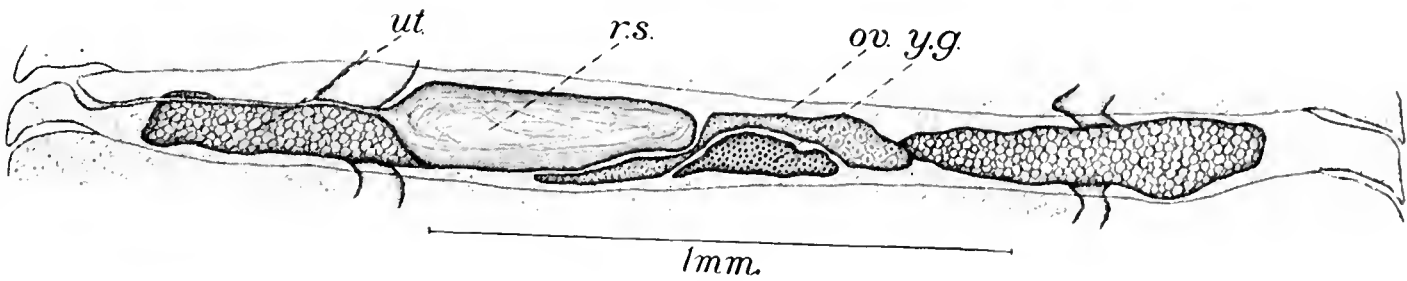
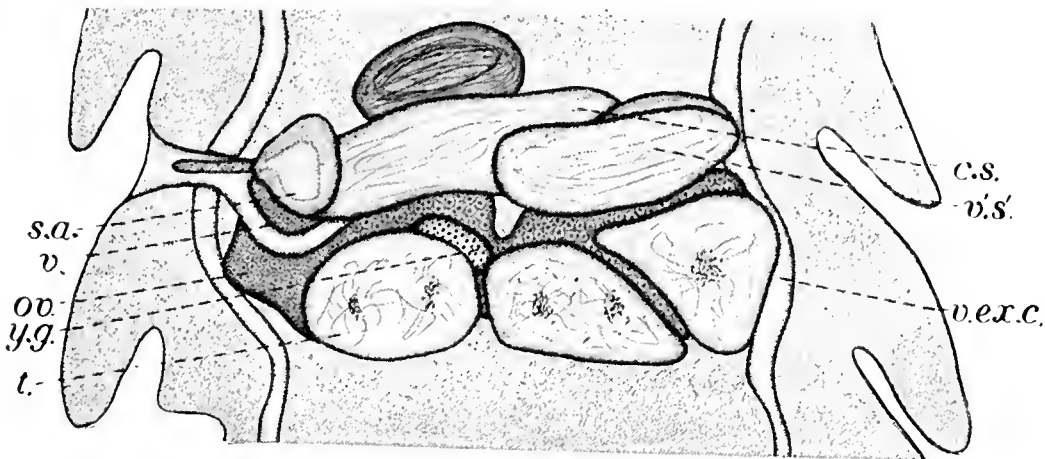


Fig. 2



0.5mm.
Fig. 3

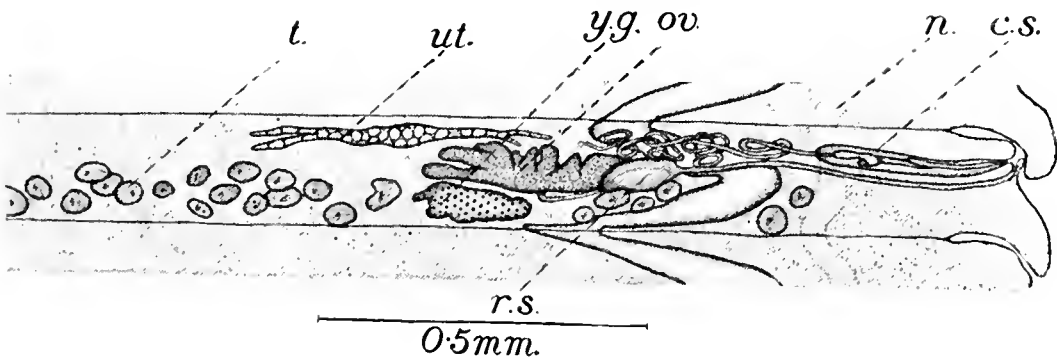


Fig. 5

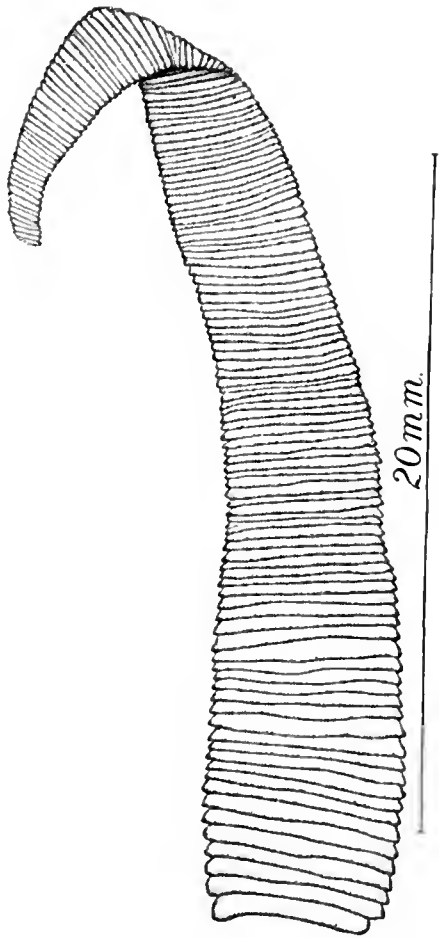


Fig. 4

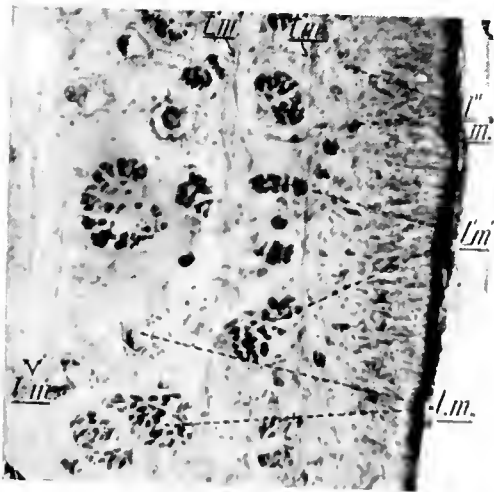


Fig. 6

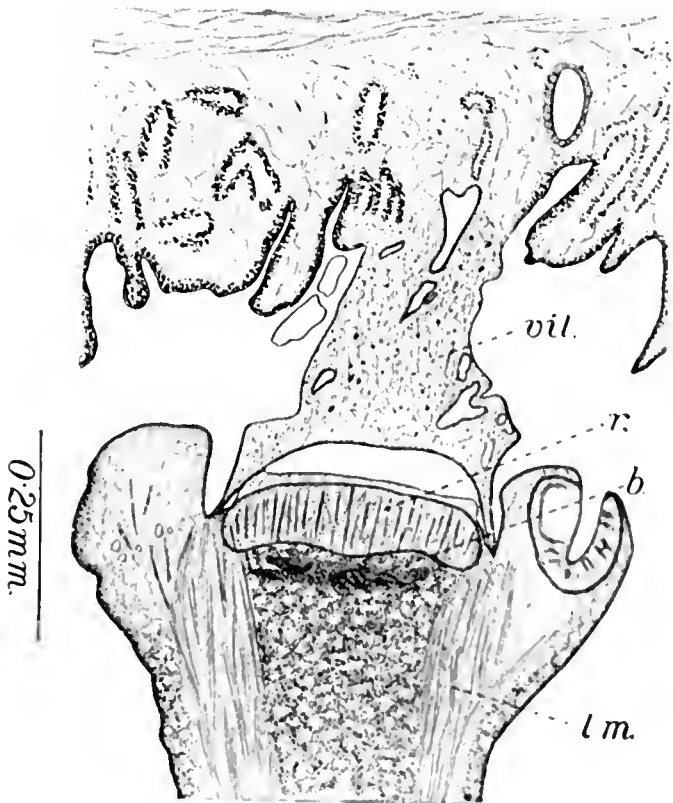


Fig. 7

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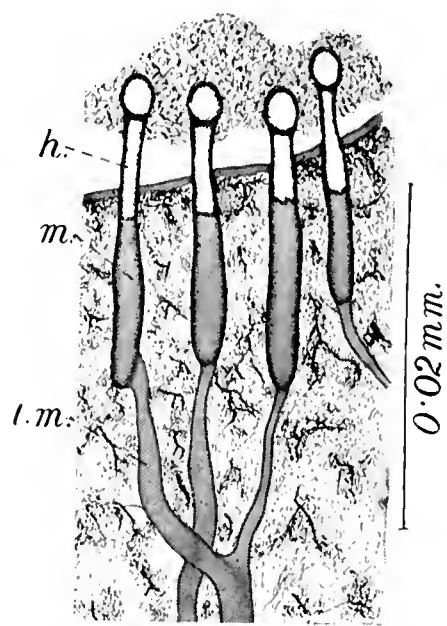


Fig. 8

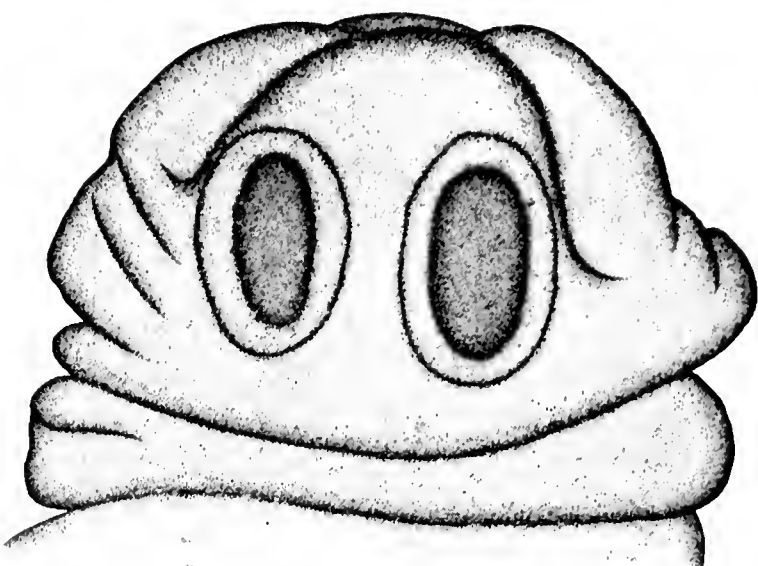


Fig. 9

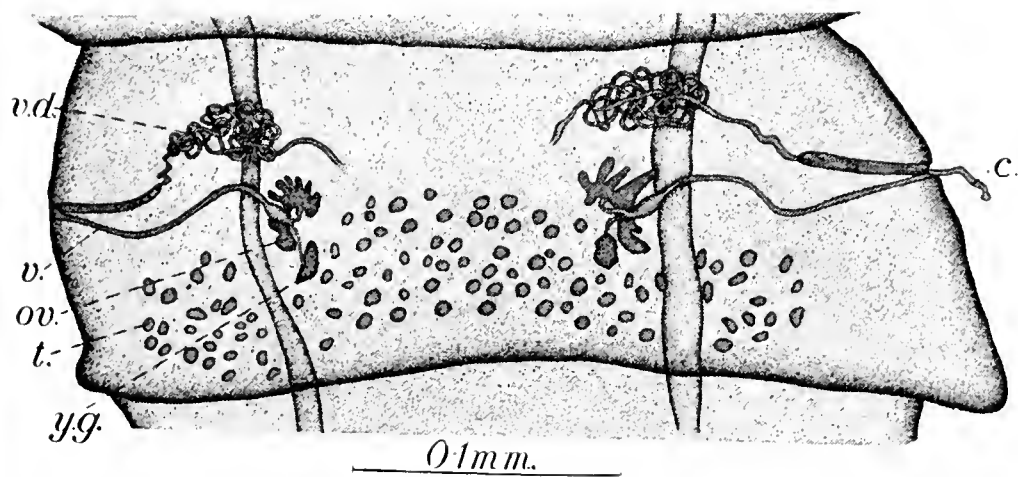


Fig. 10

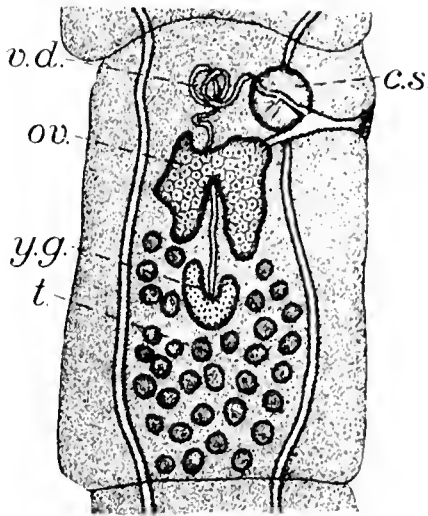


Fig. 13

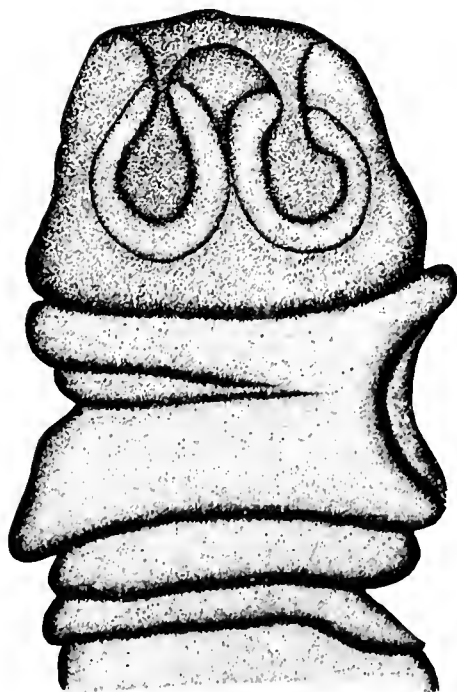


Fig. 12

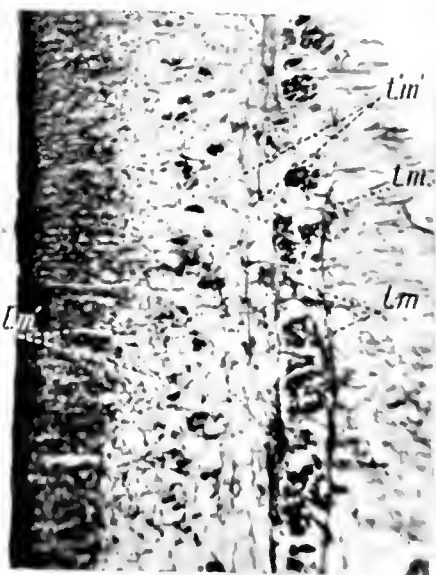


Fig. 11

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DESCRIPTION OF PLATES XVI AND XVII.

The following letters apply to all the figures:

b., central nerve mass; *c.*, cirrus; *cl.*, genital cloaca; *c.s.*, cirrus-sac; *h.*, hooks; *l.m.*, *l'.m'*, *l''.m''*, longitudinal muscles; *m.*, musculature of hooks; *n.*, longitudinal nerve; *ov.*, ovary; *r.*, rostellum; *r.s.*, receptaculum seminis; *s.a.*, sacculus accessorius; *t.*, testes; *t.m.*, *t'.m'*, transverse muscles; *v.*, vagina; *v.d.*, vas deferens; *v.ex.c.*, ventral excretory canal; *vil.*, fused villi of intestine; *v.s.*, internal vesicula seminalis; *v'.s'*, external vesicula seminalis; *ut.*, uterus; *y.g.*, yolk-gland.

PLATE XVI.

Figs. 1-2. *Hymenolepis coronula* (Duj.).

Fig. 1. Proglottis showing male organs.

Fig. 2. Older proglottis showing female organs.

Fig. 3. *Hymenolepis gracilis* (Zed.). Mature proglottis.

Figs. 4-7. *Cotugnia fastigata* n. sp.

Fig. 4. Strobilus without scolex.

Fig. 5. Half a mature proglottis.

Fig. 6. Transverse section through musculature of proglottis.

Fig. 7. Scolex attached to intestine of host.

PLATE XVII.

Fig. 8. *Cotugnia fastigata* n. sp. Hooks from rostellum.

Figs. 9-11. *Cotugnia digonophora* (Pasq.).

Fig. 9. Scolex.

Fig. 10. Mature proglottis.

Fig. 11. Transverse section through musculature of proglottis.

Figs. 12-13. *Oochoristica erinacei* n. sp.

Fig. 12. Scolex.

Fig. 13. Mature proglottis.

CORRIGENDA

Page 212, second paragraph, last line, after "equally bad" insert:

"and snails are common during the rainy season,"

Page 215, Legends to Figs. 1—4, the magnifications should be altered to read:

Fig. 1, $\times 115$; Fig. 2, $\times 230$; Fig. 3, $\times 230$; Fig. 4, $\times 485$.

ON SOME NEW HAEMOGREGARINES FROM BRITISH EAST AFRICA.

BY CECIL A. HOARE, B.Sc.

(From the Zoological Laboratory of the University of Petrograd and the National Institute for Medical Research, London.)

(With Plate XVIII.)

At the beginning of 1917 Professor V. Dogiel kindly handed over to me, for scientific treatment, a series of blood-films taken from different Amphibia, Reptilia, and Mammalia by the Expedition of Professors V. Dogiel and I. Sokolov to British East Africa in 1914. The films were examined by me for blood parasites, with the result that, of 27 animals, two different species of *Bufo* and two snakes proved to be infected. The blood parasites were represented by haemogregarines exclusively. The blood-films had been fixed on cover-slips by the dry method, and preserved in that condition for nearly three years. Altogether, I had at my disposal only 24 slips with blood-films taken from the peripheral blood of the infected animals.

For staining, I adopted the Romanovsky-Giemsa solution, Delafield's haematoxylin combined with aqueous eosin, and Pappenheim's combination of Jenner's stain and the Romanovsky-Giemsa solution. On account of the length of time during which the preparations had been preserved in a dry condition, the staining could not have been expected to produce quite favourable results. Thus, the stroma of the erythrocytes when stained with Giemsa's solution assumed a light blue, or greenish colour, instead of pink; the structure of the protoplasm in the parasites was not distinctly exhibited, their nuclei also being often hardly visible.

As regards the hosts of the parasites, it is only in one case that the specific name can be stated, namely in a Puff-adder, *Bitis gabonica*, whereas the second snake, and the two representatives of the Anura in which parasites have been found, will be defined only after the preparations of these animals are received from Alexandria, where the Expedition was compelled to leave the bulky materials collected, owing to the outbreak of war¹. In due time it is hoped that the hosts will be named in a supplementary note to this paper.

Notwithstanding all these unfavourable conditions, the material presented some interesting data.

¹ V. Dogiel and I. Sokolov. The route and brief description of the travel. *Sci. Res. Zool. Exped. to British East Africa and Uganda in 1914*, Vol. 1, Petrograd, 1916.

This work was completed in 1917 in Petrograd, but could not be published in Russia on account of conditions that resulted from the revolution. I am indebted to Professor V. Dogiel for placing at my disposal this material, and for his assistance in the treatment of it. On my arrival in England in May 1920, I had to revise this paper and bring it up to date, since we were altogether out of touch with recent scientific literature in Russia. I avail myself of the opportunity to express my warmest thanks to Professor Clifford Dobell, who has given me valuable advice in my work and provided me with all the necessary literature and accommodation at the National Institute for Medical Research, London. I am further indebted to the Medical Research Council for a grant which enabled me to proceed with this work.

1. HAEMOGREGARINES FROM SNAKES.

Host: *Bitis gabonica* (labelled *Snake No. 4*), from Mabira, June 6th, 1914.

The parasites are encountered within the blood corpuscles exclusively. The degree of infection of the latter is not great. The haemogregarine occupies not more than three-quarters of the length of the host-cell, which remains unaltered both as regards form and size. No influence of the parasite on the structure of the nucleus of the erythrocyte was observed; the nucleus is only dislocated, as is usual in haemogregarinosis.

In form this haemogregarine resembles the generic type represented by *Haemogregarina stepanowi* from a tortoise, *Emys orbicularis* (Reichenow, 1910). The body of our form is more or less bean-shaped, one end being somewhat broader than the other (Plate XVIII, figs. 2, 3). The concave side of the body, in most cases, corresponds to the convexity of the nucleus of the erythrocyte, but frequently this side is turned to the periphery of the blood corpuscle. Minchin (1907) believes that the bean-shaped form of the intracorpuseular parasite is due to its adaptation to the space limited, on one hand, by the convexity of the nucleus of the erythrocyte and, on the other hand, by the curvature of the margin of the corpuscle, and explains the reverse position of the parasite by its active movements preceding the abandonment of the corpuscle. Reichenow (1910), however, disputes this point of view and holds that the bent form of the parasite is due, not to the influence of the nucleus of the host cell, but to the structure of its protoplasm. The latter opinion is perhaps correct, as it may be frequently observed that the nucleus of the erythrocyte is displaced to one of the ends of the parasite, the concavity of which, nevertheless, corresponds to the convexity of the host nucleus.

The protoplasm of the haemogregarine is uniformly granulated, no special inclusions being visible in it. The nucleus is centrally located, occupying about one-quarter of the body length. The parasite is surrounded by a light area. The chromatin substance of the nucleus seems to be arranged in the shape of trabeculae or a network, and sometimes assumes the aspect of a glomerulus resembling a stage of division described by Prowazek (1907) in *Haemogregarina platydactyli*. Such a picture of the nucleus shows that we are

perhaps dealing with a ripe trophozoite or schizont, in which the first indications of the asexual process of multiplication are present. The average measurements of this form are 14μ in length, 6μ in breadth.

Besides this form, sometimes a second is encountered (Plate XVIII, fig. 1), which is more slender, with a smaller nucleus of more compact structure. One end of this form is always bent over in the shape of a small tail. In this form also the light surrounding rim¹ is more sharply outlined, and it is possible that it represents an earlier stage.

In form and size the haemogregarine resembles that described by Dutton, Todd and Tobey (1907), also from a Puff-adder. However, the briefness of their description, and the drawings, do not establish the identity of these two forms. Plimmer (1912) also mentions some haemogregarines found by him in *Bitis arietans*, but his description is still briefer; he characterizes them by only three words "medium, host-cell unaltered." Similar forms were described by Minchin (1910) from a snake in Uganda, but the host is unrecorded. He also examined the blood of a Puff-adder, but with negative results.

As regards the classification of the haemogregarines from snakes, Lutz (1901) considered it possible to unite them all in one species—*Drepanidium serpentium*; most authors, however, follow the principle set forth already by Simond (1901), according to which a definite species of haemogregarines is peculiar to each host species. Dobell (1908) adopts the same view for practical reasons. On the other hand, Sambon (1908) regards the haemogregarines of allied host-species as "host-varieties" or "host-races," corresponding to the "geographical races" of certain free-living animals. At present, however, the haemogregarines are still so inadequately worked out, and so few complete cycles of development are known, that it is impossible to adopt any of these views unreservedly. For practical reasons, Simond's view may be adopted provisionally.

Because neither Dutton, Todd and Tobey, nor Plimmer gave specific names to the haemogregarines found by them, and, because of the opinion just expressed by me, I propose to name the parasite herein described as *Haemogregarina dogieli*, in honour of Professor V. Dogiel.

Host: labelled *Snake No. 5* ("green tree snake"), from Mabira, June 19, 1914.

In this host the parasites are also intracorpuseular exclusively (Plate XVIII, figs. 5, 6, 7). The erythrocytes are infected in a very slight degree. The haemogregarines usually attain the length of the host cell, or are somewhat longer, slightly bending with their ends inwards. The influence of the parasitism on the host cells is distinctly visible. In my preparations nearly all the erythrocytes infected are diminished in size; as compared with the normal blood corpuscles, their cytoplasm stains very feebly, and, in some cases, there seems to be only a shadow of it visible (dehaemoglobinization). The nucleus

¹ The subject of "light areas," "rims," or capsules in general, is discussed at the end of this paper.

is, on the contrary, in nearly all cases hypertrophied and elongated parallel to the long axis of the parasite (cf. Fig. 4, and Figs. 5, 6, 7), whilst the interior structure of the nucleus loses its normal aspect. The regular rounded lumps of chromatin are no longer visible, and the chromatin mass appears to be entangled in irregular accumulations and strands. In some places vacuoles are visible (Fig. 5). In a word, indications of karyolysis are present.

Possibly in this case the diminution in the size of the erythrocyte presents already a secondary stage in the degeneration of the corpuscle, as this process usually begins with a hypertrophy of the cell and nucleus, succeeded by a diminution and shrinkage of the cell and fragmentation of its nucleus.

The haemogregarine described is disposed parallel to the long axis of the host cell, sometimes closely adjacent to the nucleus of the latter (Fig. 5). The body of the parasite is elongated, slender, never bent over on itself, only the ends may sometimes be slightly bent inwards (Fig. 5). Measurements: length 15 to 16 μ , breadth 2.25 μ ; size of normal erythrocyte 15.5 μ \times 11 μ .

The protoplasm stained very feebly in my preparations; nevertheless, it is possible to trace a slight granulation in it. The nucleus is elongated (3 μ long), more or less centrally located.

In general appearance, form of the nucleus, and action on the host cell, our parasite resembles *Haemogregarina* (*Karyolysus*) *crotali* from *Crotalus confluentus* (Sambon, 1909), but it still more closely resembles one of the stages of *Karyolysus gracilis* described by Wenyon (1908) from a lizard—*Mabuia quinquetaeniata*. Like ours, this form presents the only stage found in the peripheral blood, whereas all the other stages of the asexual cycle take place in the internal organs (liver cells). Wenyon, and subsequently Reichenow (1912), regarded this form as a gametocyte (as yet sexually undifferentiated).

Taking into account the pathological action of our parasite on the blood corpuscle infected by it, and its likeness to the representative of the genus *Karyolysus* mentioned above, it is possible to refer our form to the same genus. Although at present we are provided with a description of the complete life-cycle of one representative of this genus—*Karyolysus lacertarum* (Reichenow, 1912), which affords us an exact criterion for distinguishing between the genera *Haemogregarina* and *Karyolysus* independently of their pathological rôle—the old classification also remains in force for parasites in which the complete life history has not yet been studied. According to this classification, to the genus *Haemogregarina* are referred all forms which do not do any injury to the nucleus of the host cell, whereas all those that produce such an effect are referred to the genus *Karyolysus* (Reichenow, 1912; Doflein, 1916). Notwithstanding the close resemblance between our form and *K. gracilis*, I prefer to leave the question regarding the species of our parasite open, on account of the presence of only one stage in my preparations.

2. HAEMOGREGARINES FROM AMPHIBIA.

Host: labelled *Bufo* No. 9, from Molo, July 3, 1914.

In the blood of this host the haemogregarines are encountered in large numbers, both intracellularly and free in the blood plasma. The influence of the parasite on the erythrocyte is considerable: both the cell-body and the nucleus undergo alterations. We shall return to these changes below.

The haemogregarines are represented by a whole series of forms, evidently representing stages of the asexual cycle of multiplication. For the sake of convenience, we may divide the forms met with here into the four following types:

Type 1. Elongated slender form, thickened and rounded at one end and gradually tapering to a tail at the other (Figs. 9, 10, 11). The measurements of these forms are 25μ in length and 3μ in breadth. The nucleus is elongated, situated nearer to the blunt end, of rather compact structure. The protoplasm is granulated. These forms are encountered sometimes free in the plasma (Fig. 9) and sometimes in the act of entering or leaving the erythrocyte (Figs. 10, 11). According to the data of some authors (Stebbins, 1905) the blunt end of the parasite should be regarded as the anterior end, as it is this end that is directed forwards during progression of the parasite. Therefore Fig. 10 represents the parasite penetrating into the corpuscle, whilst Fig. 11 shows its emergence. Probably the temporary invasion of the blood corpuscle by the parasite does not remain without effect on the former. This influence is visible on comparing Fig. 8 with Fig. 11. Pictures similar to those described here from fixed preparations were observed by Stebbins (1905) in *Rana clamata* in the living state. This author states that the parasite swims in the blood plasma and "is able to enter and leave the blood corpuscles with the greatest ease and rapidity, and always mutilates the corpuscles badly in so doing." Dobell (1910) also found free forms of *H. berestneffi* (from *Rana tigrina*) which were actively motile. He "observed small forms enter red corpuscles. They did this by boring directly into the corpuscle....Occasionally, the animal, after reaching the inside of the corpuscle, rested for a few minutes and then wriggled its way out again into the blood plasma." The same observer tells me that he saw similar phenomena in the case of the haemogregarines of the rat-snake (*Zamenis mucosus*). Probably the same takes place in our case as well. This is also demonstrated by the fact that fragments of erythrocytes and their free nuclei are frequently encountered amongst intact blood corpuscles.

It should be noticed here that many authors, *e.g.* Flu (1910), Reichenow (1910), Sambon (1908), Shortt (1917), assert that the extracorporeal state of the parasite is due to abnormal conditions arising on account of the blood being exposed to the air for some time, whereas normally, and when immediately fixed, all the haemogregarines remain intracorporeal. This view is categorically refuted by Seidelin (1911), who had "seen them free in pre-

parations which were taken from the living animal and immediately fixed," and by Schubotz (1913), who observed very numerous free forms of *H. pettiti* (from *Crocodilus niloticus*) in the blood from peripheral vessels and from internal organs (liver and spleen). In fresh preparations sealed with vaseline this author kept the free forms living for hours. To this question I cannot give a definite answer based on my own material, but judging from pictures like Figs. 10 and 11, and from the complete absence of these forms within the blood corpuscles, it is easier to agree with Seidelin. The phenomena observed by Stebbins and Dobell give further confirmation of it.

Type 2 is represented by intracorpuseular forms of different size; one end of their body is much thickened, the other terminates in a short point, the body being bent over in different degrees (Figs. 12, 13, 14). The nucleus is of the usual vesicular structure, and the protoplasm is granulated, the granulation being coarser than in the preceding form. Sometimes the protoplasm contains dark staining lumps, probably of the so-called volutin (Figs. 13, 14). The blood corpuscle infected by this form is hypertrophied (Fig. 13) and its nucleus undergoes degeneration (Fig. 12).

The most numerous are forms belonging to

Type 3. All of these are intracorpuseular, the body is of an irregular bean-shaped form, measuring $18.8\mu \times 7.8\mu$. The nucleus of the parasite is disposed nearer to one of the ends of the body, the protoplasm is granular, and probably sometimes contains volutin granules (Fig. 15). This form is, moreover, characterized by the constant presence of a kind of dark cap situated on one end of the body (Figs. 15, 16). This formation evidently presents the frequently described accumulation of excretions of the parasite (Dutton, Todd, and Tobey, 1907; Stevenson, 1911; MacFie, 1914, and others).

The influence of this form of the parasite on the cell harbouring it is expressed in a general shrinkage of the erythrocyte, the body of which is ultimately reduced to the condition of a thin membrane enclosing the parasite. At the same time the nucleus of the erythrocyte degenerates and often undergoes fragmentation, breaking up into two parts (Fig. 16), as has been already frequently described by other authors (Stebbins, 1905; Plimmer, 1912; Conor, 1912; Reichenow, 1912; Shortt, 1917). This form resembles the haemogregarine described by MacFie (1914) from African toads, and, in some respects, that described by Shortt from *Bufo melanostictus*.

In all the types described above, the parasite is surrounded by a distinct light rim.

To *type 4* belong parasites whose body is club-shaped, or of irregular form, from 19μ to 34μ long, characterized by a large nucleus of round-oval form and vesicular structure (Figs. 17, 18). All forms of this type were encountered free in the plasma and only in one case a parasite was found in the act of leaving the host cell, in which the remains of the abandoned capsule are visible (Fig. 17). Similar forms were described by Dutton, Todd, and Tobey (1907), Flu (1910), and others.

As regards the mutual relationship between all the forms described above, we can, of course, only make such suppositions as are based on cycles of development already studied.

The forms of *type 1* (Figs. 9, 10, 11) doubtless represent the young stages of the parasite just beginning its cycle of development. Probably they are merozoites and pass through a certain period of free life, in the course of which they penetrate into the erythrocytes for a short time (sometimes pictures are seen in which only the middle portion of the parasite lies in the blood corpuscle, whilst its ends are free), as was stated by Stebbins (1905), Dobell (1910), Neresheimer (1909), and others. This stage is probably succeeded by an intracorpuseular stage, during which the parasites grow and bend over, adapting themselves to the limited space within the erythrocyte (Figs. 12, 13). In this period there appear in the protoplasm of the parasite the characteristic granules of volutin.

The *volutin* probably represents reserve material formed in the protoplasm and used up for the formation of nuclear substances during the maturation of the nucleus in the period of multiplication. The appearance of volutin in the protoplasm indicates the beginning of the processes of reproduction (Reichenow, 1910; Doflein, 1916).

Taking into consideration the circumstances stated above, we may suppose that the forms described as *type 2* (Figs. 12, 13) represent adult, ripe trophozoites. The form represented in Fig. 14 probably shows the stage at which one of the limbs of the body is retracted. Such a process usually precedes the formation of the schizont (Reichenow, 1910).

The forms of *type 3* are encountered in the films in predominant and very large numbers. It is to be supposed that after complete reduction of the "tail" the form just described (Fig. 14) passes to this stage (Fig. 15); as is also confirmed by the resemblance in the appearance of their nuclei (the structure of the latter is hardly discernible in these stages).

The succeeding stages of development evidently take place in the internal organs.

The forms represented in Figs. 17 and 18 are encountered only in single cases. It is possible that they represent the intermediate stages between the initial (Fig. 15) and final stages of the process of schizogony.

In the forms of *type 4* (Figs. 17 and 18) a gradual increase in the size of the cell, and a swelling of the nucleus, are visible.

Of course, all the considerations set forth here are hypothetical, as it is impossible to form a definite judgment regarding the successive phases of a complex cycle of development based on separate forms from several blood films.

As has been mentioned, some of our forms resemble, in general features, the parasites described by Dutton, Todd, and Tobey, MacFie, Stebbins, Stevenson, and Shortt. This likeness is, however, always limited to some single stage, whereas the other forms differ sharply from our parasites. More-

over, in most cases it is necessary to compare my findings with drawings alone, as the descriptions are always too short.

One of the forms that most closely resembles ours is *H. nucleobisecans* from *Bufo melanostictus* (Shortt, 1917). This form differs from ours in the presence of small forms, and in its free forms being much larger and of a different shape. *H. nucleobisecans* seems to affect the host nucleus in the same way as ours, splitting it into two in some cases. According to Shortt "this is brought about by the pressure of the concave border of the capsule of the parasite upon the adjacent border of the host-cell nucleus." In this case the fragmentation of the nucleus seems to be purely mechanical, differing in that respect from the fragmentation of the host nucleus seen in the genus *Karyolysus*.

If this be the case in our parasite, it is difficult to decide to which genus it should be referred in the absence of any sexual stages.

For the sake of registration, I propose to refer the parasite, provisionally, to the genus *Haemogregarina* and name it *Haemogregarina moloensis* nov. spec.

Host: labelled *Bufo* No. 8, from Molo, July 3, 1914.

The blood corpuscles of this animal were strongly infected with haemogregarines. The host cell does not seem to undergo any special alterations; the enlargement observed is to be ascribed to mechanical causes, being due to the expansion of the growing parasite. In some cases, however, the nucleus of the erythrocyte is seen to be broken up into two parts.

The haemogregarines occurring in the blood of this host are represented by several forms.

1. The predominant form of the parasite is a large haemogregarine of irregular oval or bean shape, in most cases not bent on itself (Figs. 24, 25), but sometimes with a distinct second limb (Fig. 22), or traces of the fusion of both limbs (Fig. 23). The average measurements of these forms are 17.5μ by 11μ . Their internal structure will be described below.

2. Besides these forms, in much fewer numbers, are encountered comparatively small parasites of pretty regular spindle or oval shape (Fig. 19), measuring 14μ by 4.7μ .

3. There are also encountered somewhat larger parasites in which one end is dilated, and the other gradually attenuates in the form of a pointed tail (Fig. 20). This extremity is usually turned to the side, but not bent over.

4. And lastly there occurs another form, in general resembling the one just described, but more massive and with the tail bent over the body (Fig. 21).

The protoplasm of the last three forms appears to be finely granulated. In the first of them (Fig. 19) vacuoles are visible at both poles (possibly artifacts). The nucleus is usually disposed nearer to one of the extremities of the body. In the second form (Fig. 20) the nuclear chromatin is arranged in rather compact lumps, whilst in the third form (Fig. 21) the nucleus occupies a larger space and its chromatin is arranged more loosely.

The structure of the body of the main form (Figs. 22, 23, 24, 25) differs considerably from that of the preceding. The protoplasm of these forms has a distinct alveolar structure and contains some kind of indefinite accumulations. The nucleus of these forms has lost its definite shape and appears in the form of minute chromatin granules disposed in different ways, but chiefly in the form of a transverse band.

A similar alveolar structure of the protoplasm was described by Nöller (1912), Reichenow (1910), Schubotz (1913), and others, in schizonts, and the arrangement of the nuclear elements in the form of a transverse band is found in parasites described by Langmann (1899), Billet (1907), and Flu (1910), also in the period of their maturation. Both these data and the configuration of the present form, as well as its dimensions, perhaps indicate that we are dealing with a full grown schizont.

As regards the mutual relations between the forms described, they present a fairly complete picture of gradual growth from the young parasite to the stage of schizont.

Form 2 (Fig. 19) indubitably presents a merozoite which had only recently penetrated into the blood corpuscle. It is exactly such a form that is described as typical for the merozoite by those authors who had the opportunity of studying the complete cycle of development of haemogregarines (Reichenow, 1910, 1912, and others). By degrees, as it grows, this form begins to elongate (Fig. 20), thicken and bend over with one end (Fig. 21); this end at length reaches the opposite extremity (Fig. 22), and the parasite assumes the shape of a U. From this moment begins the alteration in the structure of the protoplasm and the nucleus of the parasite pointing to the phenomena of multiplication. In this period both limbs begin to fuse together (Fig. 23), and, finally, the massive oval form (Figs. 24, 25) characteristic of the schizont is produced.

It is interesting to mark certain peculiarities in the disappearance of one of the limbs of the doubled parasite, usually preceding the formation of the mature schizont. This process may be effected in two ways:

1. The tail gradually shortens and appears to be drawn into the main portion of the body. This course was described by Reichenow (1910) in *H. stepanowi*, and also takes place in the haemogregarine described by me above (Fig. 14).

2. The tail bends over and grows to the length of the main portion of the body, and then both limbs fuse along the whole line of their contact. Such a course was described by Simond (1901), and Woodcock (1912). This phenomenon is also observed in the parasite just described (cp. Figs. 20, 21, 22, 23, 24).

In conclusion I shall dwell briefly upon a disputed structure common to nearly all haemogregarines, this structure having been termed by various authors "the light area" or "rim," "membrane," "cyst," and "capsule." With regard to this element various opinions have been expressed.

In most cases it is altogether ignored, or the author barely mentions that the parasite is enclosed in a capsule, without any further definition or description.

With regard to the nature of this structure the opinions of authors differ. Some (Langmann, 1899; Laveran and Pettit, 1911) regard the light area surrounding the parasite as an artificial product, due to unequal contraction of the protoplasm of the parasite and host cell under the action of fixing agents, resulting in detachment of the parasite from the cytoplasm of the erythrocyte; hence the empty space which is absent in normal ones.

The majority of authors (Prowazek, 1907; Dutton, Todd and Tobey, 1907; Dobell, 1908; Sambon, 1908; Robertson, 1908, 1910; Seidelin, 1911; Stevenson, 1911; Conor, 1912; Reichenow, 1912; Schubotz, 1913; Sergent, 1918, and others), however, state definitely that the parasite is surrounded by a special membrane or capsule. Sambon (1908) says:

"The examination of numerous species of haemogregarines...has convinced me that all the endocorpuscular forms, save the very earliest, are enclosed within a shell or capsule produced by the parasite itself, either by means of a special secretion or by a process of ecdysis, the haemogregarine shrinking from its detached former skin, as do certain flies in the formation of their puparium. The capsule varies much in size, shape, thickness, transparency and other particulars according to the species of haemogregarine to which it belongs, as well as to the stage of development....The presence of a capsule may be evidenced by a number of indications. Thus, in preparing films for microscopical examination, some of the haemogregarine-infested blood cells may be disrupted; the parasites remain closely doubled up, being evidently confined by a capsule, to the exterior of which fragments of the host cell nucleus may be seen adhering....Sometimes we may detect a second inner shell produced by a subsequent ecdysis. This inner membrane may also show deeply staining granules. After the escape of the parasite the remains of the broken capsule may be seen either still within the host cell or free in the liquor sanguinis....The capsule appears to burst along definite lines of cleavage....Two of these lines are to be seen, one at each end of the capsule....After bursting open the two halves of the capsule roll up after the fashion of the mature seed pods of certain leguminous plants."

Similar statements are made by other authors. Thus, Prowazek also found "um den in einer Höhlung der Zelle ruhenden Parasiten...eine rotfärbbare Niederschlagsmembran." Dutton, Todd and Tobey encountered in the blood of snakes and amphibia curled remains of the capsules in the form of "rods" lying in pairs. According to Dobell, Robertson and Sergent the capsule is not only distinctly visible in intracorpuscular forms, but may frequently be encountered either free in the plasma, or in the corpuscles abandoned by the parasites. Seidelin also observed delicate red lines at the extremities of the capsule (Sambon's "lines of cleavage"), and Stevenson stated that the "cyst wall" was double at the end at which the parasite is bent, the space between

the layers being filled up with a dark substance (cp. Fig. 15). Schubotz, on the other hand, declares that in the parasites described by him (from a turtle, *Cycloderma aubryi*) the capsule never appears in the form of a double-contoured membrane.

The two aspects of the question find a compromise in the views expressed by Woodcock (1912) and Shortt (1917). According to these authors, the empty space around the parasites is due to shrinkage, the parasites, however, being surrounded by a distinct thin sheath which envelops their body closely.

My preparations provide no data on which I could base authoritative conclusions. However, it is difficult to interpret the picture presented in Fig. 17 as "shrinkage" of the parasite, and I am inclined to acknowledge the correctness of the second supposition, with the amendments made by Woodcock and Shortt. The question regarding the origin of the capsule—whether it presents the product of secretion of the parasite itself, as Sambon and Woodcock think, or is formed by modification of the adjoining portion of the cytoplasm of the erythrocyte as a reaction against the influence of the parasite—remains open, although the former supposition seems to be more probable.

APPENDIX.

In several blood films from *Bufo* No. 8 and *Bufo* No. 9 were encountered bodies (Figs. 26, 27) which differ so markedly from all the blood parasites hitherto known that I suspect them to have found their way into the blood or preparations by accident, the more so, as they were found in different hosts with distinct parasites.

These bodies are spindle shaped, measuring 7.8μ by 1.5μ , the protoplasm is slightly granular, the nucleus centrally disposed, round and prominent, on account of its light colour. The structure of the nucleus is indiscernible. These bodies were nearly always encountered in groups of four in a row, as represented in Fig. 26, rarely in pairs or singly.

Once there occurred a pair of such bodies twice as large as the preceding, surrounded by a kind of capsule or membrane (Fig. 27).

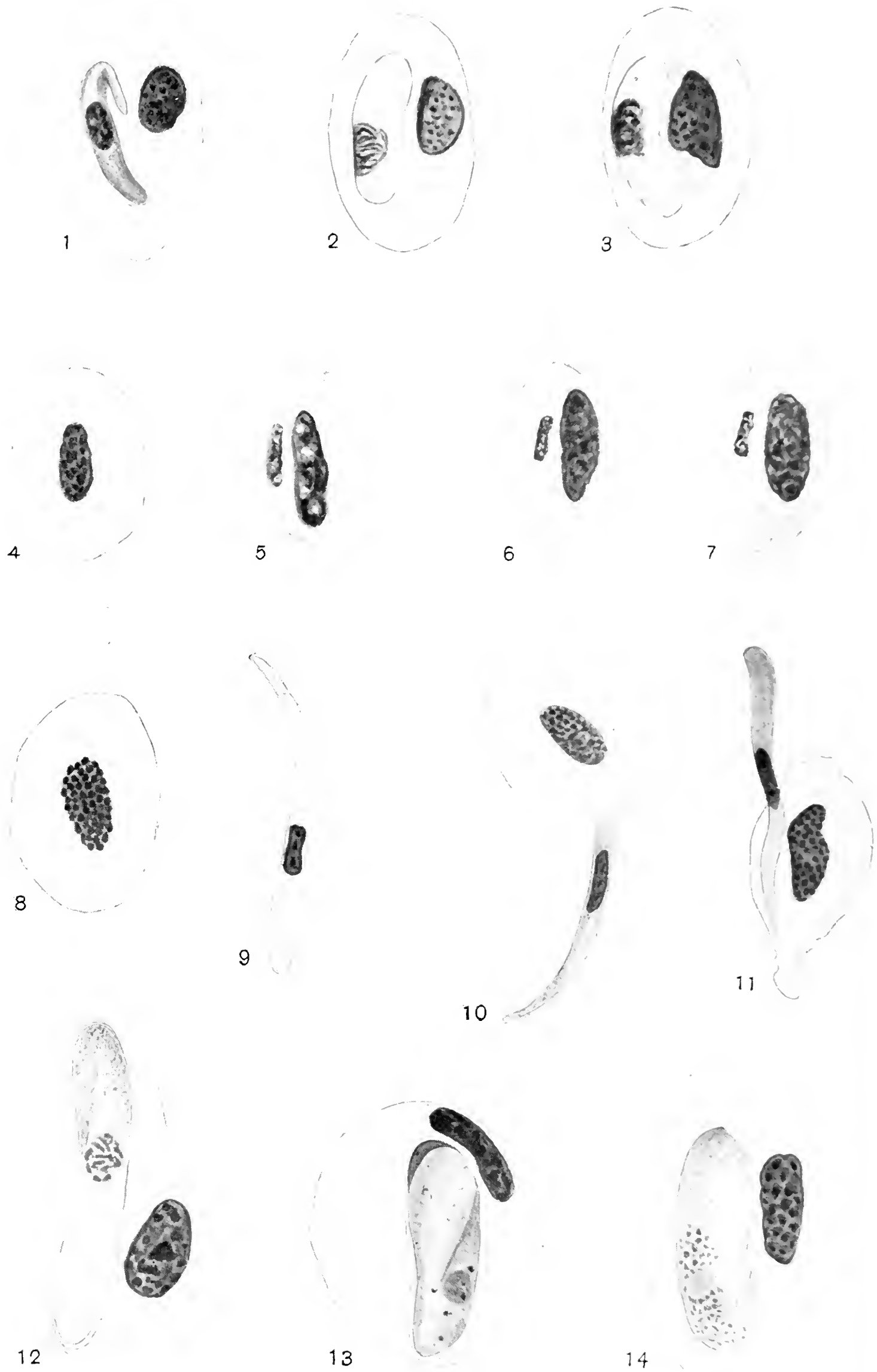
Unfortunately I can say nothing regarding the nature of these bodies. Mr C. Dobell suggested they might be the spores of an Ascomycete.

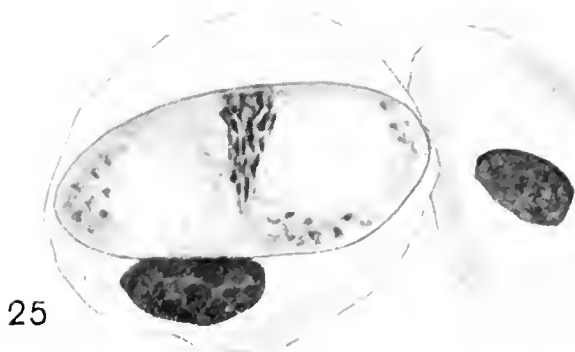
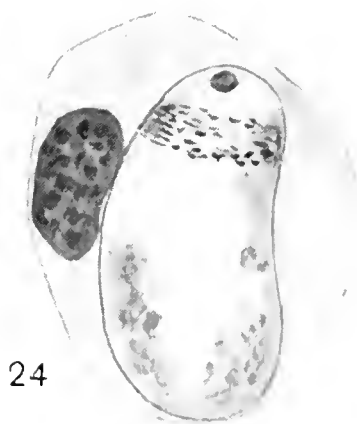
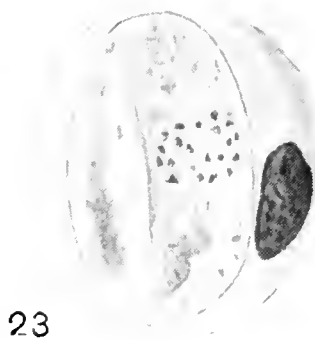
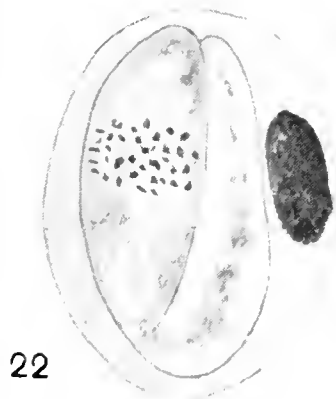
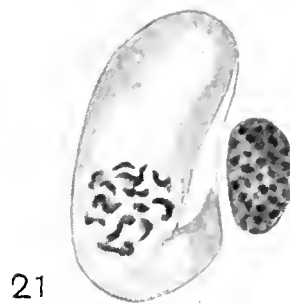
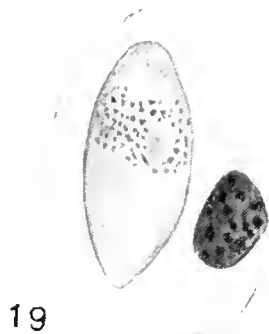
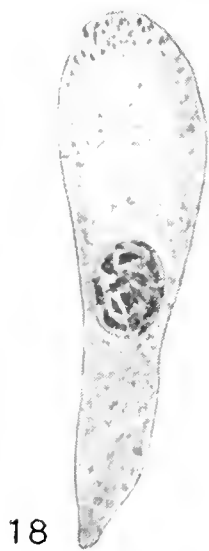
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EXPLANATION OF PLATE XVIII.

All the preparations were drawn by means of Leitz's camera lucida, at a magnification of about 1500 diameters (Leitz, oc. 4, Hom. imm. $\frac{1}{12}$ "').

Figs. 1 to 3: from *Bitis gabonica*.

Fig. 1. Small (young) form of haemogregarine.

Figs. 2, 3. Trophozoites.

Figs. 4 to 7: from a *tree snake* (No. 5).

Fig. 4. Normal erythrocyte.

Figs. 5, 6, 7. Haemogregarines. In Fig. 5 the parasite is closely adjacent to the vacuolized nucleus of the blood corpuscle. In Fig. 7 the nucleus of the latter is hypertrophied.

Figs. 8 to 18: from *Bufo* No. 9.

Fig. 8. Normal erythrocyte.

Figs. 9, 10, 11. Merozoites: free, entering and leaving the erythrocytes.

Figs. 12, 13. Trophozoites.

Fig. 14. Trophozoite, one limb of which is being reduced. Granules of volutin visible.

Figs. 15, 16. Full grown trophozoite (schizont). Volutin visible. Dark hood at one of the poles visible. In Fig. 16 the nucleus of the erythrocyte has broken up into two parts.

Figs. 17, 18. Stages of maturation of the schizont. In Fig. 17 the parasite is seen to be leaving the corpuscle in which the abandoned capsule is visible.

Figs. 19 to 25: from *Bufo* No. 6.

Fig. 19. Merozoite.

Figs. 20, 21. Same: growth and bending.

Fig. 22. Mature form (trophozoite).

Fig. 23. Same: fusion of the two limbs.

Figs. 24, 25. Schizonts.

Figs. 26, 27. Bodies of undetermined origin.

FISH MYXOSPORIDIA FROM PLYMOUTH.

BY J. S. DUNKERLY, B.Sc. (Lond.).

Natural History Department, Glasgow University.

(With 6 Text-figs.)

DURING the summer of 1914 and during the months January to April, 1920, I have been able to investigate the Myxosporidian parasites of fish occurring at Plymouth, and I am glad to take this opportunity of thanking the Ray Lankester Trustees for their grant in aid of this research, and also to express my gratitude for the very valuable and kind assistance afforded me by the Director, Dr E. J. Allen, and staff of the Marine Biological Station at Plymouth, where the researches were carried out. The assistant Mr Smith helped me very considerably in the identification of the fish examined.

I have been unable as yet to work out the material fully, especially the interesting developmental stages, and as it may be some time before I am able to do this, it may be useful to give a list of the forms found in fish met with at Plymouth, and to describe briefly some new forms.

Quite a number of new hosts were found and it is a matter of difficulty at present to decide whether a parasite found in a new host is a new species or not. The same difficulty occurs of course in other groups of parasites, notably Trematoda and Anoplura, and while some species are certainly found in many different hosts, others apparently are very restricted in their choice of hosts, even when abundant opportunities occur for infection. In this paper a very conservative attitude has been adopted with regard to possible new species. When a form has been found with spores which agreed with a published description, the parasite has been given the name of the earlier described species, although found in a different host. The plasmodial stages do not show sufficiently clear distinctions to serve for specific or even generic characteristics, except in rare cases like the pigmented plasmodium of *Chloromyxum*.

Some of the forms described under pre-existing names may be found subsequently to be distinct species, but with our present defective knowledge of life histories in this group it is better to describe the same species as occurring in different hosts rather than create new specific names which might have to be abandoned later. Some species like *Chloromyxum leydigi* have been recorded already from many different hosts, and it will be seen that a form indistinguishable from *Myxidium incurvatum* is here recorded from several different hosts. On the other hand, there appear to be some species which

are more restricted in occurrence. One case is that of *Sphaeromyxa ovata* which occurred in all of the three specimens of *Onos tricirratus* examined, but in only one of 46 specimens of *Onos mustela* which came from the same locality. In the one case in which it did occur, it was present in very small numbers only, as though it had not been able to establish a strong infection. It has been held that these spores are abnormal spores of *Sphaeromyxa balbianii*, but the spores are very dissimilar and were not found together by me. Another example of restriction in distribution is shown by *Myxidium incurvatum*, which was found in five out of 19 specimens of *Blennius pholis*, but not in one of 30 specimens of *Gobius* (various species) taken from the same rock pools. Yet *Myxidium incurvatum* is found in a large number of different hosts, and the Gobies must sometimes take up spores of this and other species. It can be suggested at least that not all species of fish are equally susceptible, and it appears likely that some species of Myxosporidia are specific to certain hosts, but their mere occurrence in different hosts cannot be taken as proof of specific difference, without some accompanying difference in form, size of spore, or other characteristic feature. Therefore it will be found that in this paper specimens have been identified as far as possible with pre-existing species, and also as far as possible with the species mentioned by Labbé in *Das Tierreich*. It would be a convenience to parasitologists if in the case of parasites a trinomial system of nomenclature for animals and plants could be used, indicating the specific character of the parasite and also the host from which it was obtained.

In the following list the fish hosts have been named for the sake of uniformity according to Dr Smitt's edition of Fries, Ekström and Sundevall's *Scandinavian Fishes*.

Host	Examined	Negative	Infected	Parasite
<i>Agonus cataphractus</i> ...	2	2	0	
<i>Anguilla vulgaris</i> ...	1	1	0	
<i>Blennius gattorugine</i> ...	5	5	0	
<i>Blennius ocellaris</i> ...	2	1	1	<i>Myxidium incurvatum</i> Thél. + <i>Ceratomyxa arcuata</i> Thél.
<i>Blennius pholis</i> ...	19	14	5	<i>Myxidium incurvatum</i> Thél.
<i>Bothus maximus</i> ...	14	13	1	<i>Myxidium incurvatum</i> Thél.
<i>Callionymus lyra</i> ...	15	0	15	<i>Myxidium incurvatum</i> Thél. (13). <i>M. incurvatum</i> + <i>Ceratomyxa arcuata</i> Thél. (2).
<i>Capros sanglier</i> ...	1	0	1	<i>Ceratomyxa lata</i> sp. n.
<i>Clupea pilchardus</i> ...	17	6	11	Plasmodium only (1). <i>Ceratomyxa truncata</i> Thél. + <i>Coccomyxa morovi</i> Léger and Hesse.
<i>Cottus bubalis</i> ...	3	0	3	<i>Ceratomyxa dubia</i> sp. n. (3). <i>Plistophora typicalis</i> Gurley in liver (1). <i>Chloromyxum quadratum</i> Thél. in muscles (1).
<i>Gadus luscus</i> ...	6	6	0	

Host			Examined		Negative	Infected	Parasite
Gadus merlangus	8	4	4		<i>Myxidium sphaericum</i> Thél. (3). <i>Ceratomyxa arcuata</i> Thél. (1).
Gadus minutus	5	4	1		<i>Sphaeromyxa longa</i> sp. n. + <i>Myxidium sphaericum</i> Thél.
Gadus pollachius	13	13	0		
Gastraea spinachia	1	1	0		
Gobius flavescens	4	4	0		
Gobius minutus	11	11	0		
Gobius paganellus	15	15	0		
Labrus (Crenilabrus) melops	4	4	0		
Labrus mixtus	1	1	0		
Lepidorhombus whiff (megastoma)	2	1	1		Plasmodia only.
Lophius piscatorius	4	2	2		<i>Ceratomyxa appendiculata</i> Thél. (?) (2). All four with <i>Glugea lophii</i> on nerves.
Merlucius merluccius	1	1	0		
Molva molva	1	0	1		<i>Ceratomyxa</i> sp.? No free spores seen.
Mustelus vulgaris	5	4	1		<i>Chloromyxum leydigi</i> Ming. Plasmodia only.
Nerophis lumbriciformis	9	9	0		
Onos mustela	46	44	2		<i>Sphaeromyxa balbianii</i> Thél. (1). <i>Sphaeromyxa ovata</i> sp. n. rare spores (1).
Onos tricirratus	3	0	3		<i>Sphaeromyxa ovata</i> sp. n.
Pholis gunnellus	1	1	0		
Platophrys laterna	2	1	1		<i>Myxidium incurvatum</i> Thél. + <i>Ceratomyxa arcuata</i> Thél.?
Pleuronectes flesus	8	7	1		<i>Myxidium intermedium</i> sp. n.
Pleuronectes limanda	15	10	5		<i>Ceratomyxa sphaerulosa</i> Thél.
Pleuronectes microcephalus	3	0	3		<i>Ceratomyxa lata</i> sp. n.
Pleuronectes platessa	8	8	0		
Ramphistoma belone	1	1	0		
Rhina squatina	3	3	0		
Roccus labrax	1	0	1		<i>Ceratomyxa arcuata</i> Thél.
Scomber scombrus	15	3	12		Plasmodia only.
Scylliorhinus canicula	5	5	0		
Scylliorhinus stellaris	5	3	2		<i>Chloromyxum leydigi</i> Ming.
Solea variegata	3	2	1		Plasmodia only. <i>Ceratomyxa</i> sp.?
Solea vulgaris	2	2	0		
Squalus acanthias	5	0	5		<i>Chloromyxum leydigi</i> Ming.
Syngnathus typhle...	5	5	0		
Trigla gurnardus	13	13	0		
Zeus faber	7	7	0		

DESCRIPTION OF SPECIES.

***Ceratomyxa lata* sp. n.** (Fig. 1). Host: *Capros sanglier*. Habitat: Gall bladder. Spore: $19\mu \times 7\mu$, crescentic in shape, ends rounded, polar capsules large, not marginal. The proportions and shape of this form distinguish it from any other, the nearest to it being *C. coris* Georg. (from *Coris julis*), which is less crescentic in form and from a different host and locality. A similar form was found in *Pleuronectes microcephalus*, and is given provisionally the same name.

Ceratomyxa dubia sp. n. (Fig. 2). Host: *Cottus bubalis*. Habitat: Gall bladder. Spore: $17.5\mu \times 8\mu$. Polar threads 30μ long. This form approaches *Leptotheca* in proportions, but sporoplasm does not fill spore, and the organism is therefore named as a *Ceratomyxa*, but is, like *C. coris* Georg. and to a less extent *C. lata*, an intermediate form between these two closely related genera.

Myxidium intermedium sp. n. (Fig. 3). Host: *Pleuronectes flesus*. Habitat: Gall bladder. Spore: $12\mu \times 6-7\mu$, broad ~-shaped like *M. incurvatum* Thél.,

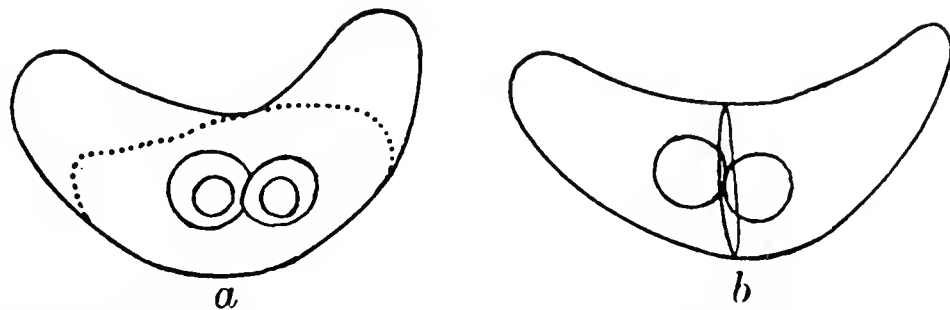


Fig. 1. *Ceratomyxa lata* sp. n. $\times 1900$. (a) Spore showing extent of sporoplasm.
(b) Spore showing sutural line.

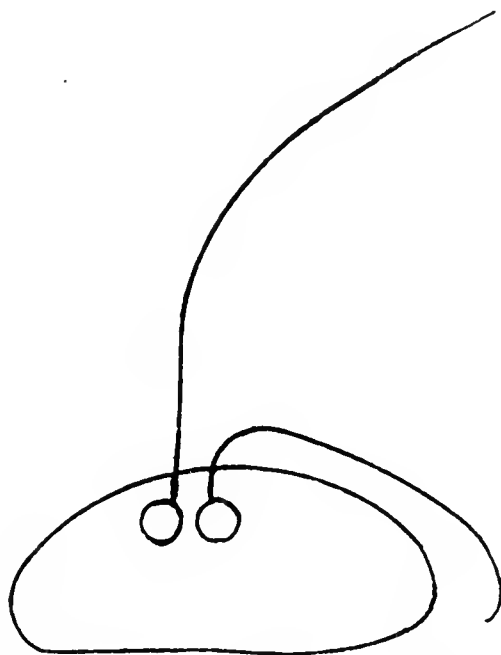


Fig. 2. *Ceratomyxa dubia* sp. n. $\times 1900$. Polar filaments extruded.



Fig. 3. *Myxidium intermedium* sp. n. $\times 1500$.

but larger and from a different host, no *Myxidium* having been recorded from *Pleuronectes*. Size alone is not a reliable guide to species as there is apparently great variation amongst specimens from different localities, though not much in any one infection. Auerbach gives the size of *Myxidium bergense* spore as $16.2-19\mu$ long $\times 7-9\mu$ wide, but specimens from *Gadus virens* caught at Millport on the Clyde, which I have carefully drawn with camera lucida and compared with a Zeiss $1/100$ mm. scale drawn under the same conditions, are all very near to the measurements $12.5\mu \times 5\mu$.

Sphaeromyxa longa sp. n. (Fig. 4). Host: *Gadus minutus*. Habitat: Gall bladder. Spore: $20\mu \times 5\mu$, similar in form to that of *S. balbianii* Thél., but much longer and from a different host. This form was compared with *S. balbianii* which was obtained from *Onos mustela* and the *S. balbianii* spores were consistently smaller, $16\mu \times 5\mu$. There was very little variation in the size of spores in either case when carefully measured, not more than 1.5μ in length. *S. longa* was found in association with *Myxidium sphaericum* Thél., the spores of *S. longa* being more numerous than those of *M. sphaericum*.

Sphaeromyxa ovata sp. n. (Fig. 5). Host: *Onos tricirratus*. Habitat: Gall bladder. Spore: $13\mu \times 6.5\mu$, oval with round ends, some slightly curved in one plane, polar capsules large, terminal. This form resembles *Cystodiscus*

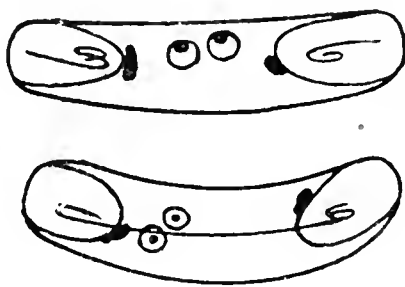


Fig. 4. *Sphaeromyxa longa* sp. n. $\times 1500$.

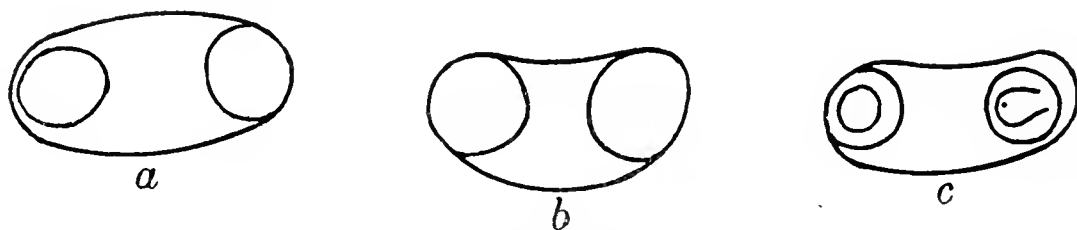


Fig. 5. *Sphaeromyxa ovata* sp. n. $\times 1900$.



Fig. 6. *Chloromyxum quadratum*. (a) Spore from above. (b) Spore with polar filaments extruded. $\times 1900$.

immersus Lutz parasitic in Amphibia, but *C. immersus* is said to be $9-10\mu$ wide. Occasionally an abnormal spore of *S. ovata* can be found measuring $10\mu \times 8\mu$, which is nearer the proportions of *C. immersus*, but is smaller. Georgevitch describes this form as a polymorph of *S. balbianii*, but no spores of *S. balbianii* were found in the three specimens of *Onos tricirratus* containing *S. ovata*. One out of 46 specimens of *Onos mustelus* showed a slight infection by this parasite, spores being very rare. This specimen was not infected with *S. balbianii*.

Chloromyxum quadratum Thél. A figure of *Chloromyxum quadratum* from the muscles of *Cottus bubalis* is subjoined to show the extruded polar filaments and the four polar nuclei (Fig. 6).

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THE CAPITULUM OF *PSOROPTES* (ACARINA)¹.

BY P. A. BUXTON, M.A.,

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(From the Quick Laboratory, University of Cambridge.)

(With 2 Text-figures.)

THIS short account of the capitulum and mouth-parts of *Psoroptes* has been prepared as a preliminary to investigations which are being made upon the bionomics of the Itch Mites. None of the published figures or descriptions are complete. The best is that of Mégnin.

The general appearance of the capitulum can be seen from Fig. 1, A and B. Viewed as a whole it is roughly pear-shaped and is inserted into the front part of the body. The dorsal surface (Fig. 1 B) is overhung by a fold of the general integument (*d.f.*, the dorsal fold or epistome), the ventral surface is free, except where it joins the body proximally. The basis capituli (*b.c.*) bears two pairs of setae on its ventral surface, a short pair arising near the palpal articulation² (*s1*) and a longer pair situated closer to the mid-ventral line. The base of the palp is so shaped ventrally that it very nearly surrounds the origin of *S1*. Both pairs of setae are directed downwards and forwards. Between the bases of the second pair (*s2*) the surface of the basis capituli bears a minute keel-like structure (*k*) which protrudes strongly in front. The distribution of the sculpturing on the ventral surface of the basis capituli is shown in Fig. 1 A. There is a roughly rectangular bare area (*b.a.*) near the insertion of the basis capituli into the body, and the sculpturing does not extend quite as far forward as the point of origin of the second seta; there is no sculpturing on the dorsal surface of the basis capituli, nor does it extend far up the side.

The mouth-parts themselves are shown in Fig. 2. I can find no difference between those of the male and of the female. The chelicerae (Fig. 2 C) are long and finely chelate. Each of the limbs which form the chela is armed with two recurved hooks (*h*), curved ventrally. The base of the chelicera is wider and more thickly chitinized than the extremity, and extends back inside the capitulum to a point slightly behind the origin of seta 2. In the dead mite

¹ Work carried out with the aid of a grant from the Ministry of Agriculture and Fisheries.

² I refer to the second appendage of *Psoroptes* as palp in accordance with the usage of most of those who have studied the Ticks and Mites. On strict morphological grounds it should be called a "pedipalp," the name which is used for the second appendage of Arachnoidea in general.

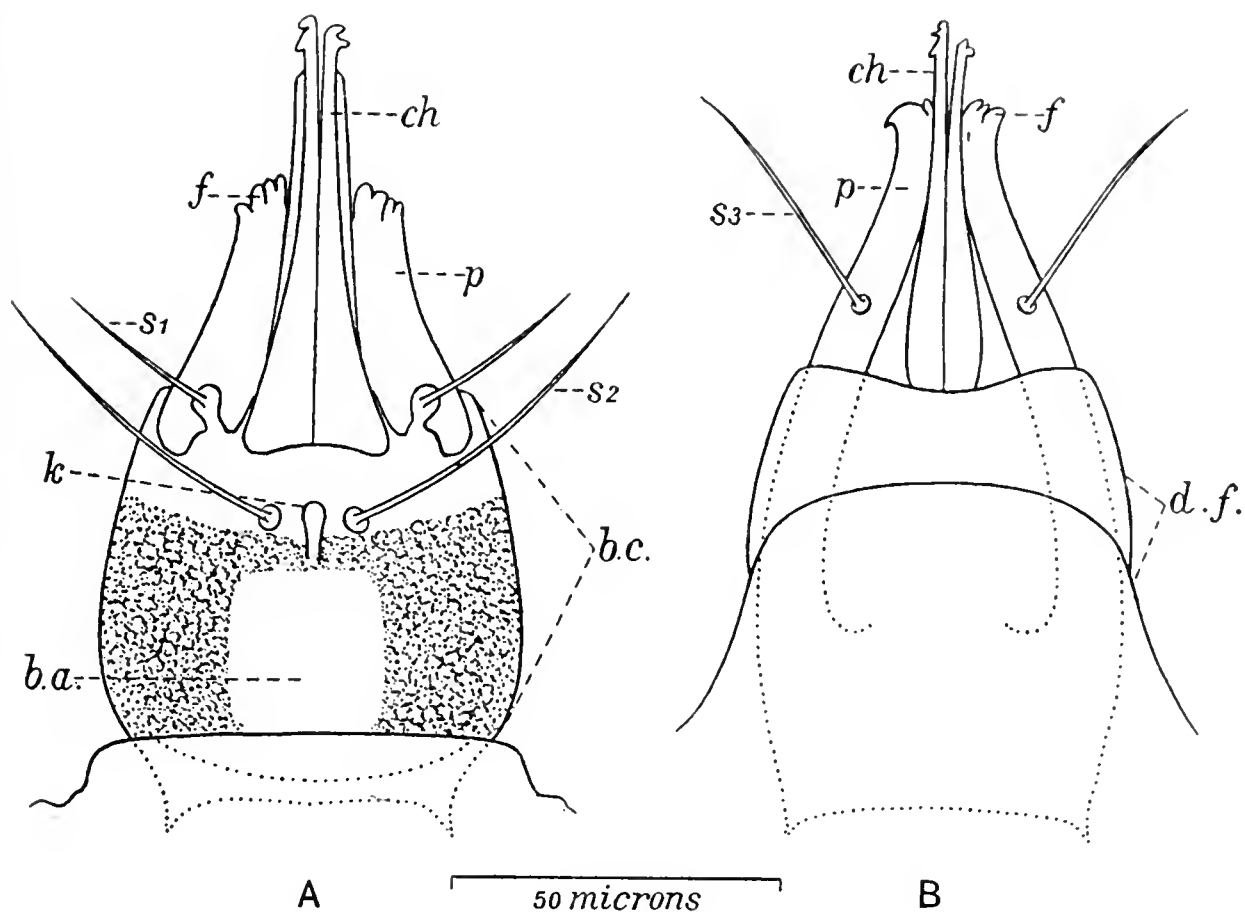


Figure 1. Capitulum of *Psoroptes equi*. A ventral and B dorsal aspects. *b.a.* bare area of basis capituli. *b.c.* basis capituli. *ch.* chelicera. *d.f.* dorsal fold of integument. *f.* finger-like processes of palp. *k.* keel-like structure. *p.* palp. *s1—3* setae. Camera lucida drawing, magnification $\times 500$.

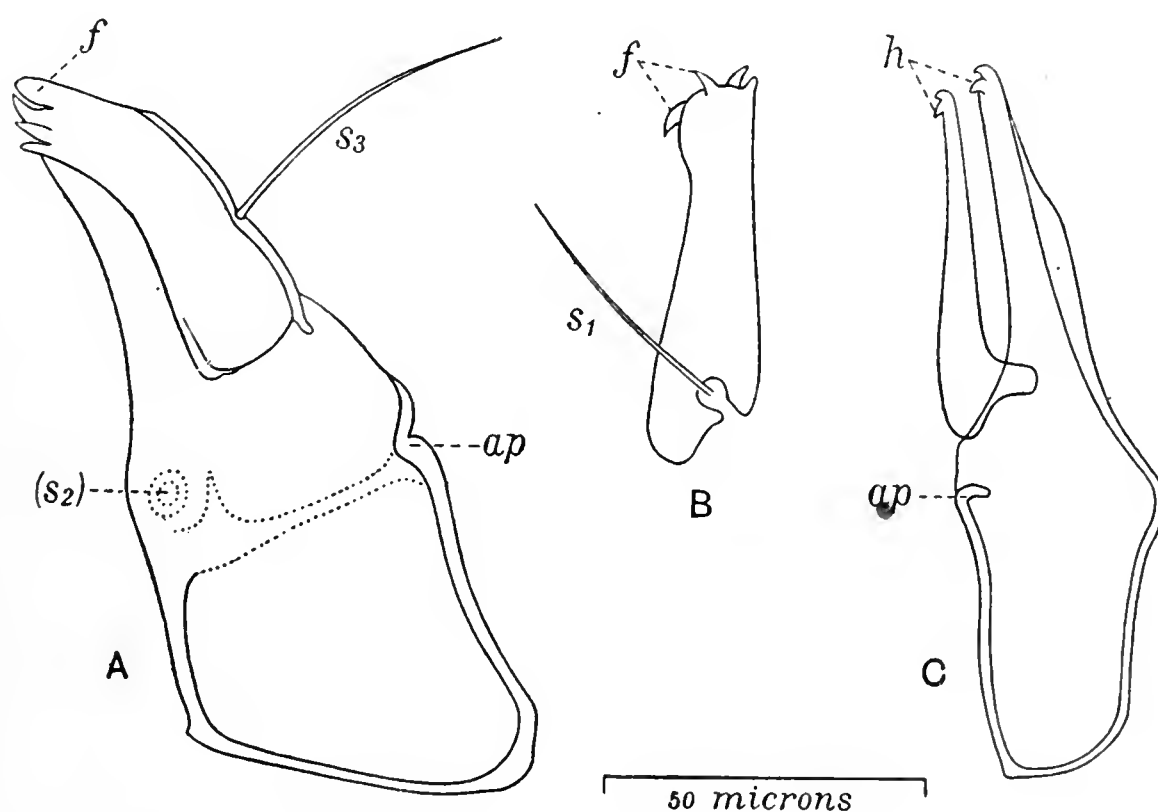


Figure 2. Mouth-parts of *Psoroptes equi*. A right palp, and basis capituli, median aspect after removal of chelicerae and left palp. B right palp, ventral aspect, with finger-like processes spread out. C chelicera seen laterally. *ap.* apodeme, for attachment of muscle. *f.* finger-like processes at end of palp. *h.* hooks on both members of chelicera. *s1* and *2* setae. (*s2*) base of second seta on ventrolateral aspect of basis capituli, seen by transparency. Camera lucida drawing, magnification $\times 500$.

one of the chelicerae is nearly always more extended than the other, but they are equal in length. The palps (*p*) arise antero-laterally from the basis capituli, flanking the chelicerae. The base of each palp is excavated (Fig. 2 B) on the ventral surface so as almost to surround the origin of seta 1, which springs not from the palp but from the basis capituli. The dorsal surface of the palp is somewhat more heavily chitinized than the rest of the organ, and from it arises a short seta (*s3*) directed forwards and upwards. The extremity of the palp is armed with about five delicate finger-like processes (*f*), covered with extremely thin, soft chitin. In some dead specimens these are seen lying side by side (fig. 2 A), in others they are spread out from the ventral face of the extremity of the palp (fig. 2 B).

I have made no observations on the manner in which these organs are used by the living mite, and it is difficult to see how such observations could be made, for not only are the chelicerae extremely small and colourless, but also the mite carries its head tucked down between the bases of the first pair of legs. It may be assumed that the mite obtains a secure hold on the skin of the host by aid of its ambulacra and tarsal claws, and that it then steadies itself by applying the finger-like processes closely to the skin. It would then bring the chelicerae into action, probably using setae 1 and 2 as organs of touch.

Mr S. Hirst of the British Museum (Natural History) has been good enough to examine some of the material on which I have worked, and says that "they seem to be *Psoroptes equi* but are not very typical. It is probable that nearly all the forms of *Psoroptes* occurring on domestic animals are merely races or varieties of a single species."

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ON A REMARKABLE NEW SPECIES OF *POROCEPHALUS* (*P. POMEROYI*, SP. N.) FROM THE FORE-GUT OF A NIGERIAN COBRA.

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College, Allahabad, U.P., India.*

*(From the Wellcome Bureau of Scientific Research, Endsleigh Gardens,
London, N.W. 1.)*

(With 1 Text-figure.)

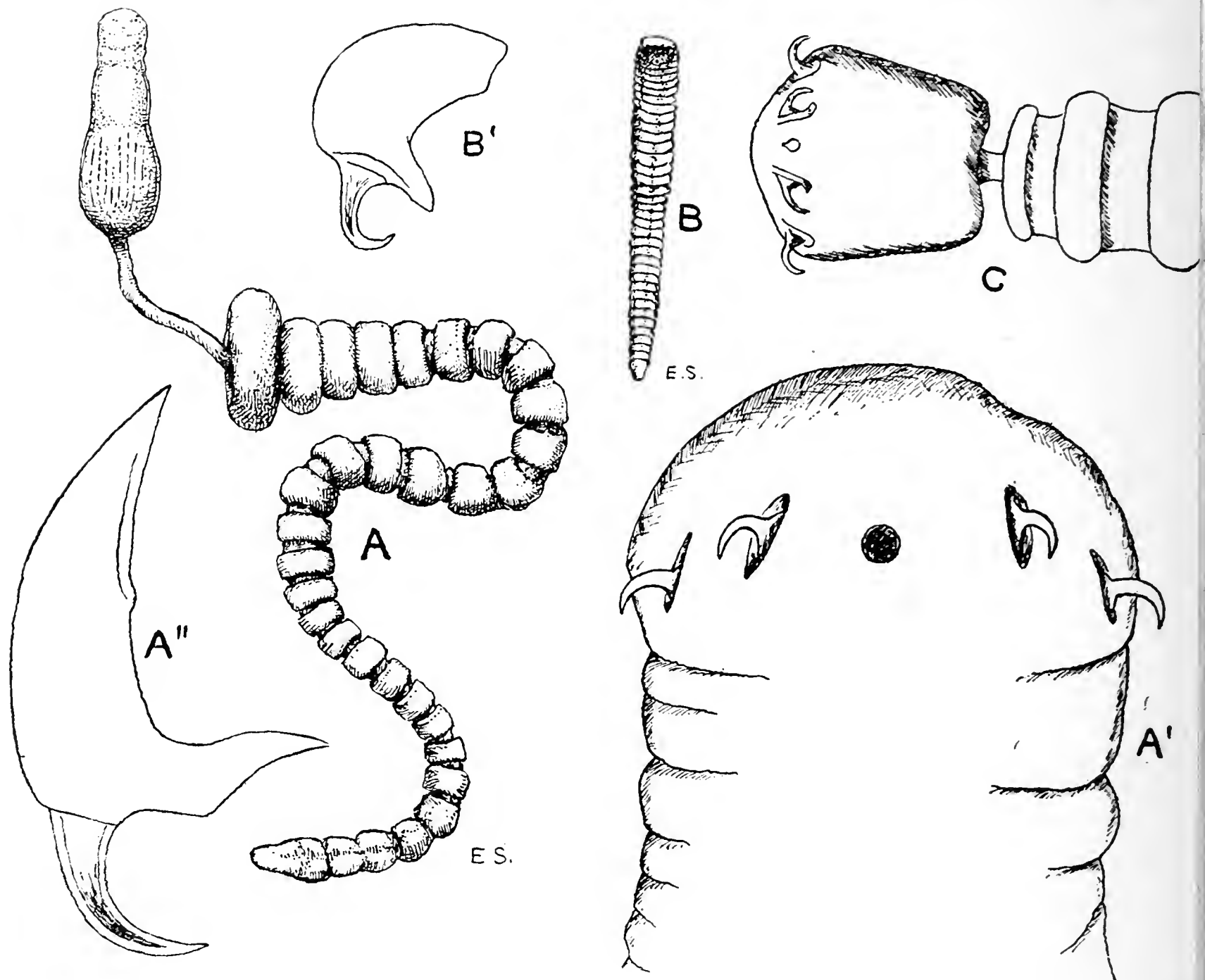
IN August, 1920, Dr Andrew Balfour, C.B., C.M.G., Director of the Wellcome Bureau of Scientific Research, received from Mr A. Pomeroy, F.E.S., Official Entomologist in South Nigeria, a tube containing some parasites from the "fore-gut" of a Cobra (*Naia nigricollis* Reinh.) from Ilaro, South Nigeria. Dr Balfour kindly handed these specimens to me for examination. The specimens were few in number, comprising only ten Nematodes and the two examples of *Porocephalus* described in the present Note. One of these examples at once attracted my attention by reason of its remarkable external form (Text-figure 1, A). I have consulted most of the available literature dealing with *Porocephalus* and the figure which, so far as I have discovered, most nearly approaches that of the present specimen is that of *P. annulatus* Baird, supplied by Shipley (his Text-figure 5, p. 59) in his memoir on the Linguatulidae¹. From my reproduction of Shipley's figure (Text-figure 1, C) it will be seen that *Porocephalus annulatus*, like the new species now to be described, has a very narrow "neck," but whereas in *P. annulatus* this neck is very short, in the new species it is comparatively very long; moreover, whereas in *P. annulatus* the cephalo-thorax (prosoma) is not longer than broad (or only slightly so in some specimens) and the first annulus of the "abdomen" (opisthosoma) is certainly no larger than succeeding annuli, in the new species the prosoma is roughly three times longer than it is broad and the first annulus is at least twice the size of the third at any succeeding annulus.

The External Features of a Female Specimen of *Porocephalus pomeroyi*, sp. n.

Text-figure 1 (A, A', A'') illustrates clearly the principal external features of a female specimen of *Porocephalus pomeroyi*, sp. n. From these I have drawn up the following diagnosis: body white, cylindrical and divided distinctly into (1) a large prosoma, (2) a long narrow "neck" and (3) an annulated

¹ Shipley, A. E. (1898), *Archives de Parasitologie*, 1, 52. Shipley provides a better figure in his article in the *Cambridge Natural History* Volume on Arachnida, p. 490, Fig. 256.

opisthosoma. In the type specimen the entire length of the body is 64 mm. The prosoma is elongated (8 mm. in the type-specimen), being one-eighth the length of the entire animal, and sac-shaped, with a maximum diameter (3 mm.) at least twice that of the opisthosoma posterior to the first two annuli. The "neck" is nearly equal in length (7 mm.) to the prosoma and in breadth



Text-figure 1. A Female specimen of *Porocephalus pomeroyi*, sp. n. ($\times 3$.)
 A' Anterior end of prosoma of the same specimen viewed ventrally. (\times cir. 27.)
 A'' Hook of the same specimen. ($\times 80$.)
 B Small male specimen of *Porocephalus* (*pomeroyi*?). ($\times 3$.)
 B' Hook of the small male *Porocephalus*. ($\times 80$.)
 C Shipley's figure of *P. annulatus*. ($\times 4$.) In Shipley's figure of this species in the *Cambridge Natural History* the prosoma is shown as being slightly more elongated.

I am indebted to Mr E. Schwarz Lenoir, artist to the Wellcome Bureau, for figures A and B.

(0.8 mm.) but little more than half the diameter of the hind portion of the opisthosoma. The opisthosoma in the type-specimen consists of 32 annuli, including the terminal or anal "segment." The first annulus is much the largest, exceeding in diameter the prosoma, and in length all the succeeding annuli. The second annulus is about two-thirds the diameter of the first

(slightly less than that of the prosoma) and is shorter. Succeeding annuli are smaller and very gradually diminish in size to the posterior extremity, the terminal annuli being about two-thirds the diameter of the anterior annuli. The anal "segment" is about twice as long as the penultimate annulus, though of the same diameter. The prosoma bears at its anterior end on the ventral surface the usual four chitinous hooks (curved, elongated, acute) and the small median sub-terminal mouth. Anus terminal. Habitat: "fore-gut" of Cobra (*Naja* sp.), Ilaro, South Nigeria. The type-specimen is a female, preserved in the collection of the Wellcome Bureau of Scientific Research.

I may remark that I could not observe any "stigmata" (orifices of epidermal glands) on the surface of the skin, nor papillae; nor could I make out the position of the female genital aperture, though, judging from appearances when the specimen was cleared in creosote, it is probably situated ventrally near the anus. The drawing of the chitinous hook was made from the creosote-cleared specimen, but the precise outline of the basal fulcrum and process was not very easy to observe. The creosote also revealed hundreds of eggs contained in the uterus extending from the sixth annulus back to the anus. Since it is desired to keep intact this one specimen of *P. pomeroyi* (the only specimen at present known to exist), I am unable to describe the internal anatomy, but this I hope to do should additional specimens be forthcoming.

*The External Features of a small Male Specimen of
Porocephalus (pomeroyi?) from the same Cobra.*

Text-figure 1 (B, B') shows the more general external characters of this small specimen, obtained from the same Cobra, and in close juxtaposition to the female *Porocephalus pomeroyi* just described. The entire body measured 12 mm. in length and consisted of the small conical prosoma bearing the usual hooks and mouth, and 37 annuli (not clearly indicated in the figure), including the small pointed anal "segment." The body is slightly flattened in the region of the first ten annuli. The hooks are as shown in the figure. When cleared in creosote the specimen was seen to be a mature male. The male aperture was situated anteriorly and ventrally at the level of the third annulus.

In general characters and in size the specimen somewhat resembles the figure of *Porocephalus aonyces* Macalister, provided by Shipley (*loc. cit.* Text-figure 6), but is probably not identical with that species, which is parasitic in the peritoneal cavity of the large Indian Otter (*Aonyx cinerea*), is 17–20 mm. in length (sex supposed to be female) and has 30 annuli.

*On the Possible Relationship between the two Specimens of
Porocephalus above described.*

Mr Pomeroy remarks in his letter concerning these two specimens of *Porocephalus* that "they seem to be in coitu"—a suggestion which I presume was based on the facts that they were of opposite sexes and that they were in close proximity in the "fore-gut" of the Cobra. Other evidence in favour

of this suggestion is to be found in their relative dimensions. The female specimen (64 mm.) was slightly more than five times as long as the male. This is also the case in other species of *Porocephalus*. Thus Spencer¹ states that in *P. teretiusculus* the female is nearly five times as long as the male; Miss Hett² similarly mentions that in *Raillietiella* a female measures 40 mm. and a male 10 mm.; Harley³ says that the largest female specimen (found in the lungs of an Egyptian Cobra) of "*Pentastoma annulatum*" was $4\frac{3}{4}$ inches long and a male (found in the nasal fossa of the same Cobra) $1\frac{1}{8}$ inch long; in *P. lari*, Mégnin, the female measures 6 cms. and the male about 1 cm., and in most other species of *Porocephalus* the female is at least twice as long as the male.

Against this view that these two specimens are a female and male of the same species are the facts that they are of very different shape externally, that the female has 32 annuli and the male 37, and that the hooks are perhaps slightly different in form. However, the exact number of segments which compose the opisthosoma cannot be regarded as a safe character on which to found specific distinctions, nor are minute differences in shape of the hooks of more value, and I think it is highly probable that the two specimens of *Porocephalus* above described are the female and male forms of the same species. If this be so, then these two specimens present the most marked form of sexual dimorphism yet discovered in the Linguatulidae.

Note on the Habitat of Porocephalus pomeroyi.

By "fore-gut" I assume Mr Pomeroy to mean the anterior part of the intestine, and not the mouth-cavity, throat or oesophagus of the Cobra. This assumption is borne out by the fact that with the two *Porocephalus* individuals ten Nematodes (the smallest 12 mm. long) were found. I need hardly point out that this situation, if correctly stated⁴, is an unusual one, the vast majority of Linguatulids being found either in the frontal sinuses, nasal cavity, trachea, lungs or body-cavity or in the substance of certain organs (muscles, liver, kidney, spleen) of Vertebrates other than fishes.

Postscript. (8. x. 1920): Mr Pomeroy informs me that the two specimens of *Porocephalus* above described "appeared to be joined together by a ligament and were not separated when I removed them from the snake." Mr Pomeroy encloses a rough sketch from memory of the way in which the two specimens were united, from which it would appear that a filamentous connection extended between the posterior sexual aperture of the large female and the exterior sexual aperture of the small male; this connection seems to prove that the pair belong to the same species.

Mr Pomeroy also confirms his previous statement that the pair were found in the intestine "about the middle of the snake, and not as I have found them before in the first part of the fore-gut."

¹ Baldwin Spencer (1893), *Quart. Journ. Micr. Science*, xxxiv, 1.

² Hett, M. L. (1915), *Ibid.* lxi, 185.

³ Harley, G. (1857), *Proc. Zool. Soc. London*, Part 25, p. 115.

⁴ Wyman (*Bost. Soc. Nat. Hist.*, Sept. 17, 1845) mentions *Porocephalus armillatus* as occurring in the intestines of a Python.

ON SOME FILARIID PARASITES OF CATTLE AND OTHER RUMINANTS¹.

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(With 7 Text-figures.)

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INTRODUCTION.

THIS memoir presents the results of the study of a number of specimens of Filariid worms from Cattle and other Ruminants which had accumulated during the last few years from various collections of Nematodes, sent to me for identification and laid aside for further investigation.

I am indebted to Professor G. H. F. Nuttall, F.R.S., for two of these collections: (1) Helminths from Wassein, Burma, sent by Dr H. H. Marshall, and (2) from British East Africa, collected by Mr R. E. Montgomery, of the Nairobi Veterinary Pathological Laboratory. A third collection was obtained from Northern Rhodesia by Mr H. E. Hornby, Government Veterinary Surgeon, whilst my material also includes some specimens sent by the Department of Agriculture, Reduit, Mauritius.

The Filariids from these various sources all proved to belong to the group of species now separated from the genus *Filaria* s. str. and placed by Railliet and Henry (1911) in the genus *Setaria* Viborg 1795. Altogether thirteen species have so far been referred to this genus, of which ten have been recorded as parasites of Ruminants; of the latter only two are at all well known, namely *Setaria labiato-papillosa* (Aless.) and *S. equina* (Abildg.), the second species essentially a parasite of Equines but stated to have been observed in Cattle also (Stossich, 1897). The remaining forms from Ruminants have for

¹ In the absence of the Author the proof sheets of this paper were passed by the Editor.—G.H.F.N.

the most part been recorded on single occasions only and the majority are very incompletely described, so much so as to be practically unrecognisable.

The material at my disposal was found to consist of four species, two of these are described as new, the others referred to the species *S. labiato-papillosa* (Aless.) and *S. digitata* (v. Linst.). Whilst describing the two new forms I have considered it advisable to add a brief account of the latter species also, since even the commoner parasite of Domestic Cattle is very shortly described in the text-books and very few records of the measurements of the various organs seem available. I have also added a short diagnosis of the genus *Setaria*.

Genus SETARIA Viborg 1795 (non Oken 1815).

Hamularia Treutler 1795, Stiles 1907.

Filaria auct. p.p.

GENERIC DIAGNOSIS. *Filariidae*: Body cylindrical, filiform, considerably attenuated at the posterior extremity in both sexes. Cuticle finely striated transversely. Mouth surrounded by a chitinous peribuccal ring, notched laterally and usually also dorso-ventrally so as to give the impression of two or four projecting teeth. Four submedian head-papillae always present, a pair of lateral papillae probably always occur as well but are frequently difficult to see. Oesophagus consisting of two parts: a short, narrow anterior ("vestibule" of some authors) and a much longer and thicker posterior region.

Male smaller than the female, its attenuated caudal extremity ending in a close spiral. Preanal and postanal papillae present as well as in the majority of species a pair of small lateral appendices close to the posterior extremity. Spicules very unequal, the longer consisting of two parts the posterior of which is largely membranous, the shorter curved.

Female with caudal region coiled in a loose spiral and bearing a pair of lateral appendices close to the extremity. Vulva near the anterior end of the body. Eggs thin-shelled. Ovoviviparous.

Parasitic in the peritoneal cavities of Mammals.

Type Species: *Setaria equina* (Abildgaard 1789), Railliet and Henry, 1911.

***Setaria labiato-papillosa* (Aless. 1838).**

This common parasite of Ruminants is represented in my material by specimens from Domestic Cattle and the Bush-Buck in British East Africa and from the Stag (*Cervus hippelaphus*) in Mauritius.

SPECIFIC DIAGNOSIS. *Setaria*: Body tapering gradually to the anterior extremity. Head rounded, not separated from the remainder of the body.

Peribuccal ring prominent, much elongated dorso-ventrally and deeply notched laterally in such a way as to give the appearance in a side view (Text-fig. 1A) of strong dorsal and ventral teeth separated by a wide depression from the centre of which arises a semi-circular lip-like elevation. Each tooth is in its turn indented so as to form two cusps, visible when the head is

viewed from the dorsal or ventral surface (Text-fig. 1B), they are usually better developed in the females than in the males.

Submedian head-papillae small, lateral papillae very inconspicuous.

The anterior region of the oesophagus measures 0.6–0.85 mm. in length, whilst the posterior region has a length of 6.5–7.3 mm.

Male: 40–46 mm. in length with a maximum thickness of about 0.6 mm.

The tail region is coiled in a close spiral, the cloaca situated 0.19–0.23 mm. from the posterior extremity. The latter is provided with a rounded terminal

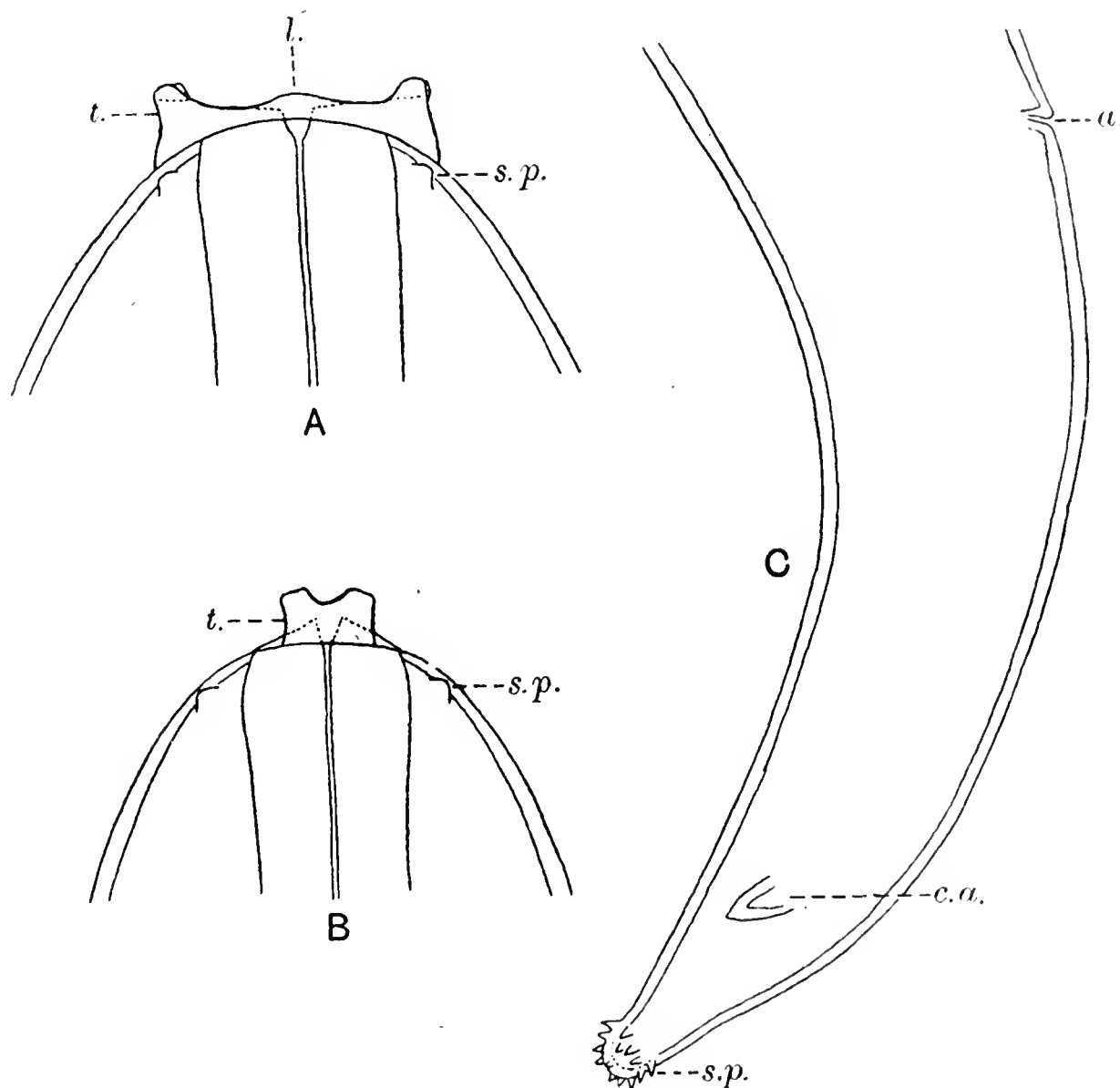


Fig. 1. *Setaria labiato-papillosa* (Aless.). A. Head in lateral view. B. Head in dorsal view. C. Lateral view of caudal extremity of female. $\times 220$.

knob close in front of which are situated a pair of conspicuous lateral appendices (Text-fig. 2). There are four pairs of preanal and four pairs of post-anal papillae.

The spicules are very unequal, the larger measuring 0.33–0.37 mm. in length and consisting of a long cylindrical anterior region followed by a much shorter terminal region which is largely membranous. The shorter spicule (Text-fig. 2) is stout, slightly curved and terminates in a sharp point.

Female: 62–94 mm. in length, with a maximum breadth of 0.7–0.8 mm.

The caudal region is coiled in a loose spiral and terminates in a knob-like extremity surrounded by an irregular ring of pointed spines, the latter very

variable in number (Text-fig. 1 C). The lateral appendices of the tail are large and situated about 0.12 mm. from the posterior extremity.

Anus about 0.5 mm. from the posterior end of the body.

Vulva somewhat variable in position, 0.45–0.75 mm. from the anterior extremity.

Eggs thin-shelled, measuring $0.03\text{--}0.046 \times 0.02\text{--}0.03$ mm.

Setaria digitata (v. Linstow 1906).

v. Linstow's type-specimens were obtained from *Bos indicus* in Ceylon; I have referred to this species a number of worms collected by Dr Marshall

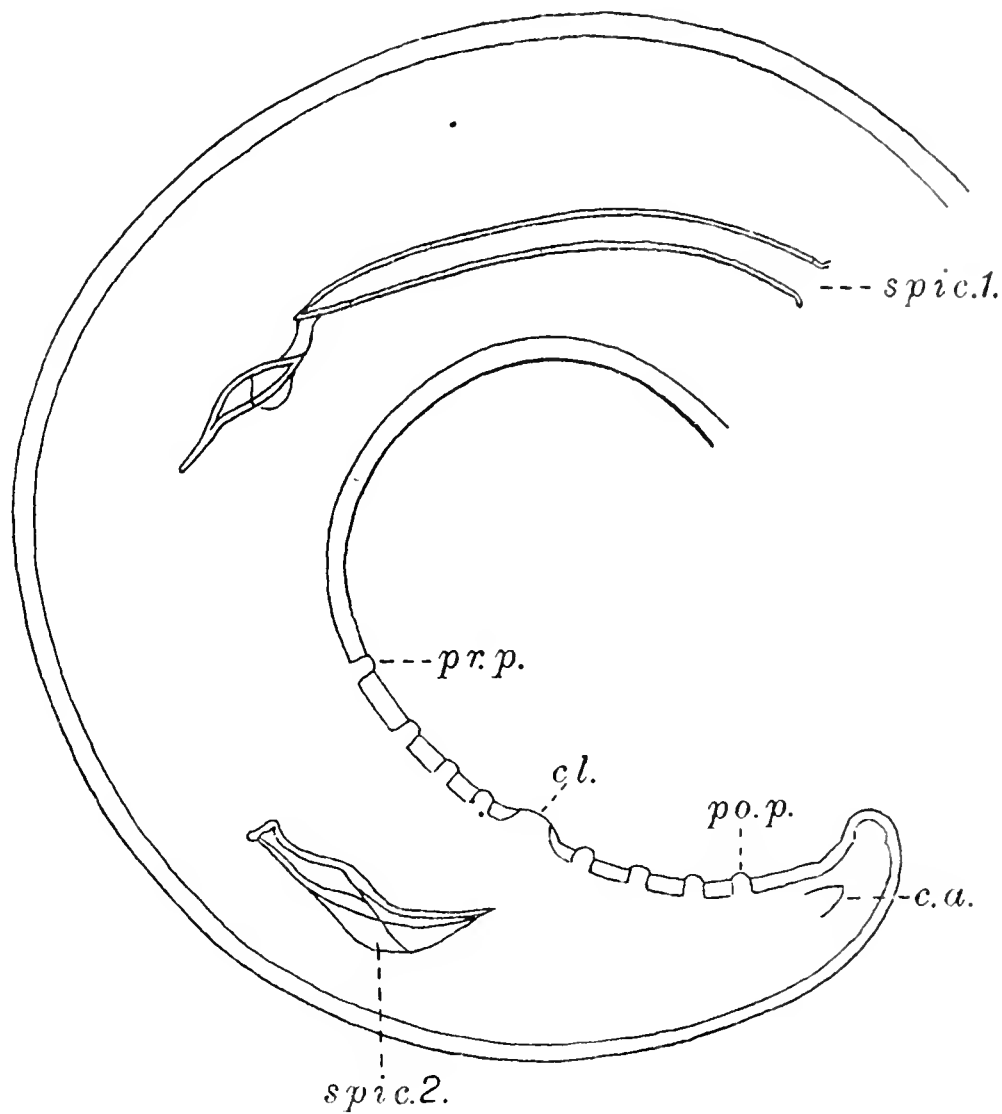


Fig. 2. *Setaria labiato-papillosa* (Aless.). Lateral view of caudal extremity of male. $\times 220$.

from the peritoneal cavity of Domestic Cattle at Wassein, Burma. Unfortunately only female specimens are represented in the material; these agree fairly well in their measurements with those described by v. Linstow. I have however given a somewhat different interpretation to the structure of the organs surrounding the mouth.

The species is evidently very closely allied to *S. equina* (Abildg.), the type species of the genus.

SPECIFIC DIAGNOSIS. *Setaria*: Head not separated from the body which tapers gradually to the anterior extremity.

Peribuccal ring resembling that of the preceding species, the lateral notches are however less deep and the lip-like chitinous projections from their centres are better developed and frequently almost triangular in shape (Text-fig. 3 A), in which case they have the appearance of lateral teeth. The dorsal and ventral teeth are notched so as to present two cusps (Text-fig. 3 B).

Sub-median papillae small and rounded, lateral papillae were not seen.

Female: 62–78 mm. in length, maximum breadth of the body 0.5–0.68 mm.

The anterior region of the oesophagus measures 0.53–0.8 mm. in length, the posterior region 6.5–8 mm.

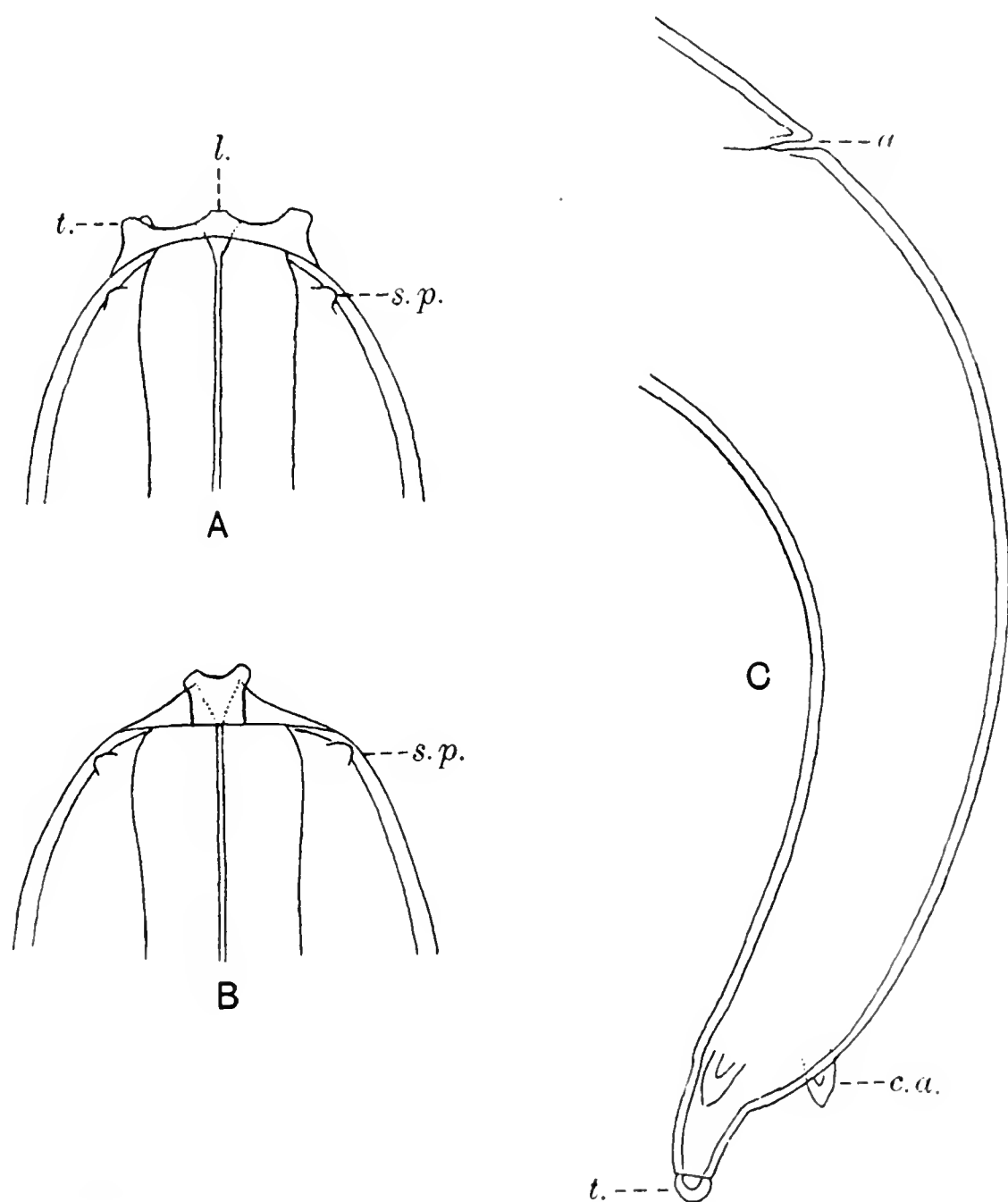


Fig. 3. *Setaria digitata* (v. Linst.). A. Head in lateral view. B. Head in dorsal view. C. Lateral view of caudal extremity of female. $\times 220$.

The posterior extremity of the loose caudal spiral terminates in a smooth knob, a pair of large lateral appendices are situated 0.1 mm. from the end of the body (Text-fig. 3 C).

Anus 0.4–0.48 mm. from the tip of the tail.

Vulva 0.5–0.65 mm. from the anterior extremity. Eggs thin-shelled, 0.032–0.045 mm. \times 0.018–0.02 mm.

***Setaria Marshalli* sp. n.**

Among the specimens of *S. digitata* collected by Dr Marshall from Cattle at Wassein, Burma, was a single female *Setaria* which differs markedly from all known forms of the genus and which I take as the type of a new species, named after the collector. The specimen was unfortunately not in a good state of preservation and the description is therefore incomplete in a few particulars.

SPECIFIC DIAGNOSIS. *Setaria*: Head not separated from the rest of the body and appearing truncated anteriorly.

The peribuccal ring is elongated in the dorso-ventral direction and is very deeply notched laterally. The lip-like lateral projections described in *S. digitata*

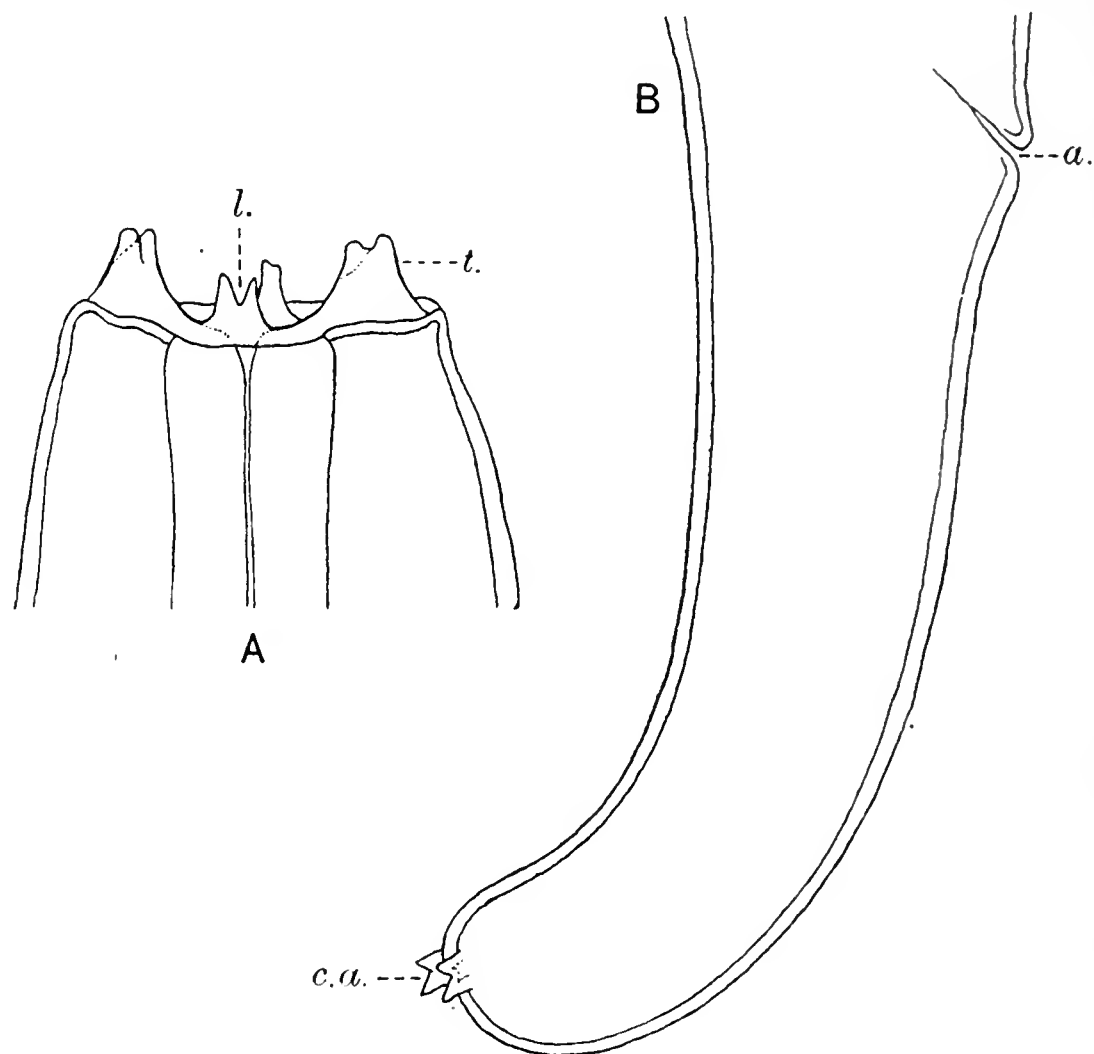


Fig. 4. *Setaria Marshalli* sp. n. A. Head in lateral view. B. Lateral view of caudal extremity of female. $\times 220$.

and *S. labiato-papillosa* are in this species exceptionally prominent and markedly tooth-like, being themselves indented at their apices (Text-fig. 4 A); they bear a strong resemblance to the dorsal and ventral teeth which are strong and provided with two cusps.

Head papillae were not observed.

Female: 90 mm. in length, with a maximum breadth of about 0.6 mm.

The oesophagus has a total length of 9 mm.

Anus 0.45 mm. from the posterior extremity, the latter is rounded and slightly curved in the dorsal direction (Text-fig. 4 B). The lateral appendices are bifurcated and situated very close to the posterior extremity of the body.

Vulva about 0.6 mm. from the anterior extremity.

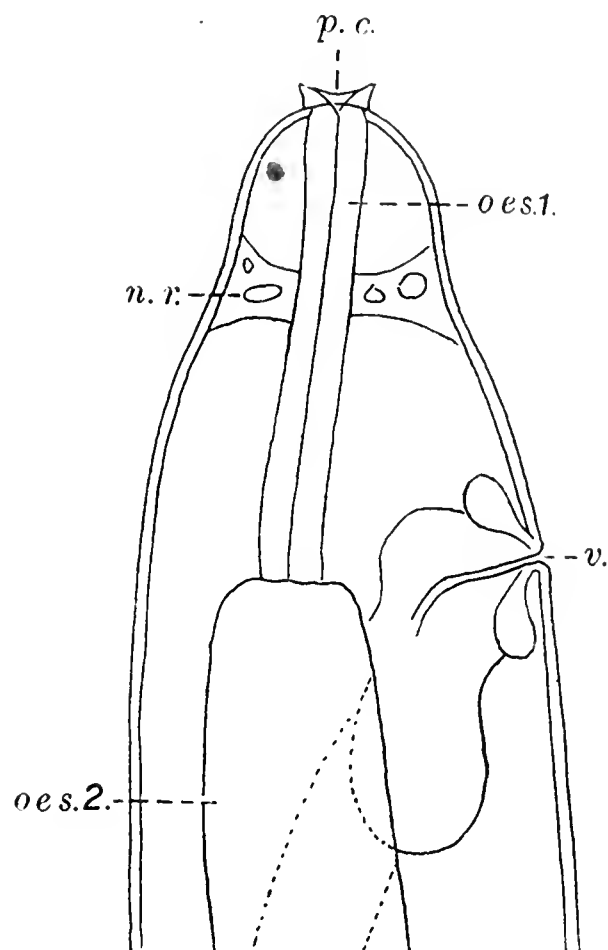


Fig. 5. *Setaria Hornbyi* sp. n. Anterior extremity of female. $\times 55$.

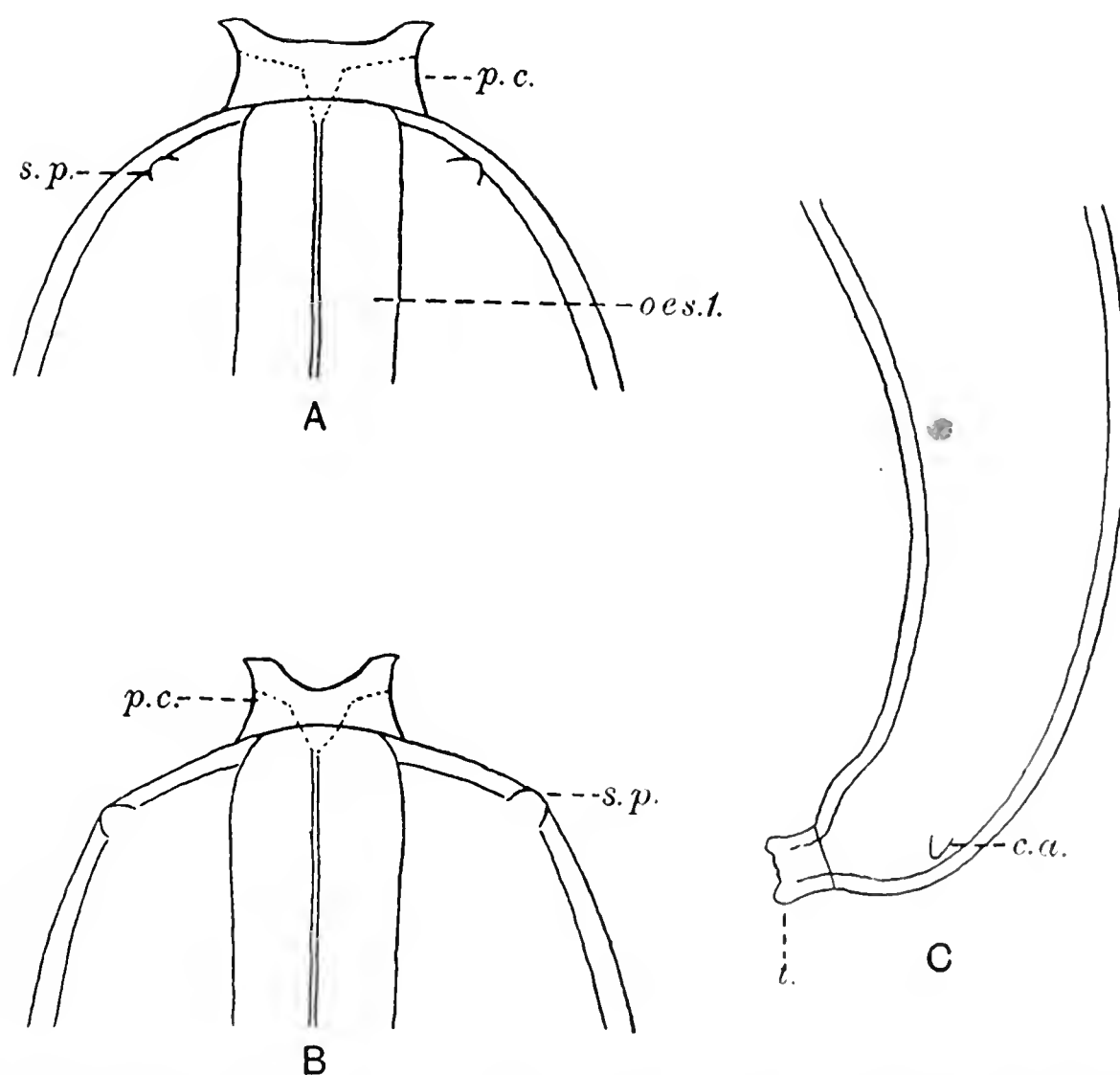


Fig. 6. *Setaria Hornbyi* sp. n. A. Head in lateral view. B. Head in ventral view. C. Caudal extremity of female. $\times 220$.

***Setaria Hornbyi* sp. n.**

Male and female specimens of a large species of *Setaria* were obtained by Mr H. E. Hornby from the Sable Antelope, *Hippotragus niger*, in Northern Rhodesia. The worms were found on the surface of the liver, in the mediastinal

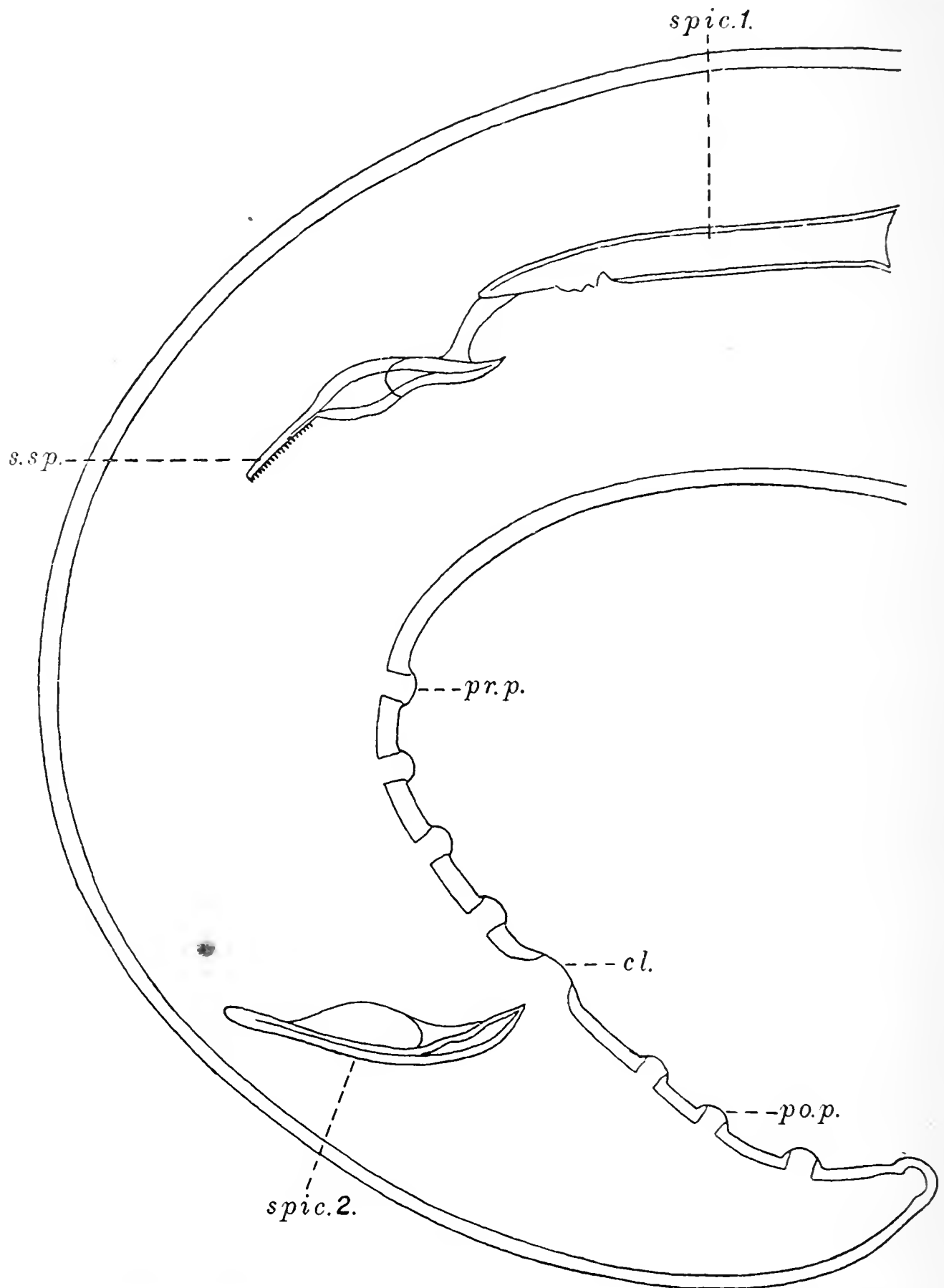


Fig. 7. *Setaria Hornbyi* sp. n. Lateral view of caudal extremity of male. $\times 220$.

fold and in the peritoneal cavity. Mr Hornby writes that he has seen similar forms parasitic in the Eland, Water-Buck and Reed-Buck.

SPECIFIC DIAGNOSIS. *Setaria*: Anterior end of the body narrowing very gradually to a point about the level of the nerve-ring, in front of which it

tapers sharply to the oral extremity. The comparatively small head is separated from the rest of the body by a slight neck-like constriction (Text-fig. 5).

Peribuccal ring in the shape of a short oval, its dorso-ventral axis being only slightly longer than the lateral; dorso-ventral and lateral notches of approximately equal depth so that the buccal armature gives the impression of a shallow ring with four truncated tooth-like processes projecting anteriorly in the submedian planes (Text-fig. 6).

Submedian and lateral head-papillae small.

Anterior and posterior regions of the oesophagus measure 0.63–0.7 mm. and 9.5–10.3 mm. respectively.

Male: 108–110 mm. in length with a maximum breadth of about 0.5 mm.

Tail region narrow, coiled in a close spiral and terminating in a rounded knob. Lateral caudal appendices were not observed and are probably absent in this species (Text-fig. 7).

Cloaca about 0.28 mm. from the posterior extremity. Four pairs of pre-anal and three pairs of postanal papillae.

Spicules very unequal, the longer having a length 0.45–0.47 mm. and consisting of an anterior cylindrical region, 0.25–0.27 mm. long and a posterior partly membranous region the terminal portion of which is finely serrated (Text-fig. 7).

Female: 200–260 mm. in length, the body having a maximum thickness of 1.25–1.5 mm.

Anus 0.8 mm. from the posterior extremity which is provided with a sucker-like terminal appendage (Text-fig. 6). A pair of small lateral appendices are situated 0.05–0.08 mm. from the extremity.

Vulva 0.7 mm. from the anterior end of the body.

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EXPLANATION OF LETTERING TO TEXT-FIGURES.

a.—anus of female; *c.a.*—caudal appendices; *cl.*—cloaca of male; *l.*—lateral lip of peribuccal ring; *n.r.*—nerve-ring; *oes.1.*—anterior region of oesophagus; *oes.2.*—posterior region of oesophagus; *p.r.*—peribuccal ring; *po.p.*—postanal papilla of male; *pr.p.*—preanal papilla; *s.p.*—submedian head-papilla; *s.sp.*—serrated end of spicule; *sp.*—ring of caudal spines; *spic.1.*—long spicule of male; *spic.2.*—short spicule; *t.*—dorsal tooth of peribuccal ring; *tc.*—terminal knob of caudal region; *v.*—vulva.

OBSERVATIONS ON THE INTESTINAL PROTOZOA OF THREE EGYPTIAN LIZARDS, WITH A NOTE ON A CELL-INVADING FUNGUS.

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Scientific Research.*

(With Plates XIX and XX, and 2 Text-figs.)

WHILE I was in Egypt conducting investigations into the etiology of dysentery with Captain F. W. O'Connor at Alexandria in 1916 I was able to make some observations on the intestinal protozoa of three species of lizard. The occurrence of an *Entamoeba* and a *Chilomastix* (*Tetramitus*) in one of these, *Agama stellio*, was mentioned in our publication on the *Human Intestinal Protozoa in the Near East*, p. 147 (1917). The other lizards investigated were *Chamaeleon vulgaris* and *Lacerta agilis*. I will describe the observations on the protozoa under their respective hosts.

1. CHAMAELEON VULGARIS.

The only protozoon found in the gut of the chamaeleon was a flagellate of the leptomonas type and it was confined almost entirely to the cloaca, though sometimes it extended in small numbers into the rectum. The only previous record of such a flagellate in the cloaca of the chamaeleon is in a paper by Bayon (1915), who discovered it in *Chamaeleon pumilus* in Robben Island in 1914. I had the opportunity of examining Dr Bayon's preparations and there can be no doubt that the measurements he quotes are erroneous. The length of the flagellate bodies is given as 25–75 μ ; breadth 2–10 μ ; diameter of trophonucleus 2.5–4 μ . In none of his preparations did I see flagellates of this size, nor have I found them in the parasite of the Egyptian *Chamaeleon vulgaris*. Evidently some mistake in calculation was made and the measurements are at least three times what they should be.

Structure of the flagellate.

The organism (Pl. XX, Fig. 1) as it occurs in the Egyptian lizard has the usual leptomonas structure—an elongate, flattened and pointed body, a central nucleus and a terminal and anterior kinetoplast with a long flagellum directed forwards, in which direction the flagellate progresses. The kinetoplast, which in many individuals is laterally placed (Fig. 1, *o*), as in other organisms of this

group, consists of a dark-staining granule, the parabasal, in front of which is a paler and smaller granule, the blepharoplast or centrosome from which the rhizoplast, continued into the flagellum, actually arises. Between these two granules, and also frequently behind them, is a clear area. Whether the whole structure represents a true nucleus of which the dark granule is the karyosome and the limits of the clear area a nuclear membrane on which the centrosome lies, as in the nucleus of *Cercomonas*, is still a matter requiring elucidation. I have brought forward some evidence in favour of the true nuclear nature of this structure in a paper entitled "Observations on *Herpetomonas muscae domesticae* and some Allied Flagellates" (1913). Most observers, however, seem to regard the structure as not being a nucleus and I quite agree there are many arguments against this view. Kofoid (1915) has suggested the abandonment of the name kinetonucleus and with it the binucleate conception of this group of flagellates. According to him the darkly staining granule is a parabasal body, a term first used by Janicki (1911), and homologous with similar structures which are associated with the flagellar origin in many other flagellates (*Trichomonas*, *Chilomastix*, *Giardia*, etc.) and which occurs in the flagellate *Prowazekella lacertae* considered below. The blepharoplast is the centrosome and leads the way in division. It must be admitted, however, that in division the behaviour of the centrosome and the subsequent division of the darkly staining mass by elongation and constriction, evidently under the influence of the centrosome, bears a striking resemblance to the division of the trophonucleus of these flagellates or of the nucleus of *Cercomonas*, as I have shown elsewhere (1913). Whether there is a nuclear membrane or not surrounding the clear area in which the darkly staining granule lies is largely a matter of interpretation, for one is dealing with such tiny objects that it is exceedingly difficult to determine this point with accuracy. In such a decision one is naturally influenced by what one has observed in larger flagellates. On account of the doubt surrounding the true nature of this structure it is better to avoid any term implying a nuclear nature so I have adopted the name kinetoplast, first suggested, I believe, by Alexeieff. The use of this word avoids the necessity of such ponderous names as kinetonucleus and trophonucleus. The kinetoplast includes both the parabasal and the blepharoplast. In degenerate trypanosomes in which the cytoplasm has disappeared the kinetoplast may remain as a compact body showing the parabasal surrounded by what appears to be a membrane on which the blepharoplast lies. The flagellum may or may not remain still attached to the blepharoplast.

As a rule the cytoplasm of the organisms is quite clear. In some, however, darkly staining granules are present, but these are probably dependent upon the state of nutrition.

The length of the body of the largest individuals (Pl. XX, Fig. 1, *f* and *m*) is about 15μ and the flagellum is slightly longer than this. The width of the body of these long forms is under 3μ . From these there may be traced a series of gradually diminishing individuals of a great variety of shape and size, as shown

in Fig. 1. Finally, very minute forms (Fig. 1, *a* and *b*) more or less spherical or circular in outline are seen, having a diameter of about 2μ . These minute flagellates have relatively long flagella. Some of them are devoid of flagella (Fig. 1, *b* and *e*) and from them small ovoid bodies with a more definite outline appear to arise (Fig. 1, *d*). These are probably encysted forms such as occur in the similar flagellate of the flea, *Pulex irritans*. I have shown in this case that complete drying of the spread-out flea faeces for 24 hours does not prevent a culture being obtained when introduced into N.N.N. medium (1912). Between these tiny parasites and the elongated form every intermediate stage can be easily traced (Fig. 1, *g*, *i*, *j*, *k*). In the small forms the nucleus and kinetoplast lie close together.

Multiplication takes place in the usual manner by longitudinal fission after division of the nucleus and kinetoplast. The new flagellum is formed by an outgrowth from the daughter blepharoplast (Fig. 1, *h*, *l*, *n*).

I have been quite unable to make out any connexion between the kinetoplast and the nucleus, nor have I seen any indication of an axostyle.

Distribution of the flagellate.

The flagellate was found in all the chamaeleons examined—about six. As already stated, it was confined almost entirely to the cloaca into which the rectum and ureters open. It was encountered in small numbers in the rectum, but not in the ureters, while in the cloaca itself it was limited almost entirely to the surface of the mucosa where it occurred in the mucus in enormous numbers. When the lizard defecates it passes a cylindrical mass of faeces or solid white urine which is covered with this mucus in which swarms of flagellates can be found. In the actual urine itself they do not occur and in the faeces only in small numbers. It is probable that the latter are forms which have passed down from the rectum. The mucus from the cloaca is a clear transparent substance and is remarkable in that there is practically no contamination with bacteria or with faecal material. In stained smears no bacteria were seen and the only organism met with apart from the flagellate was the fungus described below, which was present on two occasions.

Careful examinations of the various organs of the body both by smear and cultural methods failed to reveal any tissue infection. This is perhaps surprising in the light of the work of the Sergents, Lemaire and Senevet (1914) and the later work of Chatton and Blanc (1918). These observers have shown that cultures of leptomonas can be obtained by inoculating N.N.N. medium with the blood of the gecko, *Tarentola mauritanica*. Examinations of smears of the blood and organs failed to reveal any leishmaniform parasites, the flagellates only being demonstrated by the culture method. It seems probable that the leishmaniform bodies seen by Chatton and Blanc (1914) within the red blood corpuscles of the gecko on an earlier occasion have no connexion with the leptomonas obtained by them later in cultures. In the light of these successes it would be interesting to make further attempts to isolate a lepto-

monas from the blood and tissues of the chamaeleon, for it would seem that in this animal the intestinal infection may indicate the path by which the blood infection of the gecko was acquired.

The exact relation of the flagellates of the chamaeleon to the mucosa is best studied in sections. It is found that the infection is a very large one and that all the glands of the cloacal mucosa have their ducts packed with the organism (Pl. XIX, Fig. 1). No trace of invasion of the cells could be found, so it would appear that the flagellate is limited to the ducts of the glands and the surface of the mucosa. Fig. 2 shows a single gland more highly magnified and though many goblet cells are present there is no tendency for the flagellates to make their way into these. There was no evidence that the flagellate in any way inconvenienced its host.

Source of the infection.

As was suggested by Bayon (1915), the most probable source of infection would appear to be some insect which the chamaeleon has eaten. Flies could very readily infect themselves by feeding on the mucus covering the faeces passed by the chamaeleon, which in its turn could be infected by eating an already infected fly. Such a hypothetical cycle would seem to be the probable one.

The contents of the stomachs of chamaeleons were examined with a view to identifying the flies on which they had fed, but little information was obtained, chiefly through lack of time to follow the observations properly. However, some experiments were conducted with the ordinary house-fly, *Musca domestica*. Pupae were collected and it was found that the flies hatching from these were free from flagellate infections. A batch of these hatched flies was fed on the infected mucus from the chamaeleon's cloaca and kept alive by feeding on sugar and water. Another batch was used as control and kept alive in a similar manner.

24. VI. 16. Flies fed on infected mucus.

25. VI. 16. Several flies examined.

1. Stomach with large infection of very active flagellates.
2. Ditto.
3. Active flagellates in stomach and rounded forms in intestine.
4. Active flagellates in both stomach and intestine.
5. Doubtful forms seen.
6. Ditto.
7. Rounded forms in intestine.
8. Active flagellates in stomach and round form in intestine.
9. Ditto.
10. Active forms in stomach and intestine.

26. VI. 16. One fly had resting forms in intestine.

30. VI. 16. Four flies all negative.

1. VII. 16. Thirteen flies all negative.

None of the flies in the control experiment showed any infection.

It is evident therefore that the flagellates taken up by the flies can survive for at least two days, and the character of the infections in the flies resembled a natural fly infection, but the fact that the flagellates eventually disappeared would seem to indicate that the house-fly is not the true host of the chamaeleon flagellate.

Unfortunately time did not permit of the investigations being carried any further.

Nomenclature.

The flagellate of the chamaeleon is undoubtedly of the leptomonas type and it is of especial interest as being the only known leptomonas which is parasitic in the gut of a vertebrate. In addition to the flagellates of the gecko described by the French observers others have been recorded from vertebrates, apart of course from the well-known natural leishmania infections of man, dog and cat. The Sergents (1907) recorded their observation of a leptomonas in the blood of the pigeon. Fantham and Porter (1915) say they saw a leptomonas in mice but Mesnil (1915) is doubtful of the correctness of this observation. Balfour (1916) also states that he and Archibald saw such a flagellate in the gerbil in the Sudan. Dutton and Todd (1902) claimed to have seen this flagellate in Gambian house mice (sp. ?), but in a subsequent examination of the films Todd (1914) found the flagellate to be in reality a trypanosome which he identified with *Trypanosoma acomys* (Wenyon). *Trypanosoma lewisi* is a very active flagellate and occurs in such a variety of forms that when seen only in the fresh unstained blood it is quite easily mistaken for a leptomonas and it seems probable that the forms described by the various observers noted above were in reality *Trypanosoma lewisi*. In any case the descriptions are so meagre that it is impossible to identify the flagellates. In 1919 Marcel Leger described a flagellate (a leptomonas) which he named *L. Henrici* from the blood of two out of thirty lizards (Genus *Anolis*, Fam. Iguanidae) examined in Martinique. The organisms were typical leptomonas forms measuring 15–16 μ in length and 3–4 μ in breadth. The flagellum was longer than the body. Rarely were rounded leishmania forms seen. He subsequently found that over half the lizards examined harboured what was apparently the same leptomonas in the rectum. It would appear, therefore, that the only cases of leptomonas infections of the blood and tissues of animals are those of the N. African gecko and the S. American *Anolis*. In 1909 Knuth recorded the finding of a leptomonas in the heart blood of a roebuck but, as the animal was partly devoured and had fly larvae in its lungs, the infection may have been of extraneous origin.

On the other hand, it has been demonstrated, chiefly by Laveran and Franchini and Fantham and Porter, that many insect flagellates of the leptomonas and crithidia types are inoculable into mice and other animals and even bring about their death. These inoculation experiments are of the greatest interest from the point of view of the spread of leishmaniasis, and the French

observers (1914) have suggested that the flagellate of the gecko may in reality be *Leishmania tropica*, and that it is transmitted to man by the *Phlebotomus* which feeds on the lizards. On account of their importance it would be well if the inoculation experiments were repeated by other observers.

In the present state of our knowledge it is difficult to name many of these flagellates. Some time ago (1913) I outlined a scheme for the classification of these flagellates in the following manner. There is a group, confined entirely to the insect or invertebrate host, which in its most highly developed stage has the leptomonas structure. Small round leishmaniform bodies, protected by what must be a cyst wall, are developed from the leptomonas forms and escape in the insect's faeces. They are ingested either by the larvae or adults of the insect and lead to their infection. There is thus only a single invertebrate host and such forms can be distinguished, at any rate at present, by the generic name *Leptomonas*. This name was first employed by Kent (1881) for a flagellate seen by Bütschli in 1878 in a nematode worm *Trilobus gracilis*. Kent named it *Leptomonas bütschlii*, but whether it is a leptomonas as we now understand it cannot be determined till this organism is re-examined in the light of present knowledge. Meanwhile we shall employ the name *Leptomonas* as above defined.

Another group of flagellates, in which the highest stage of development is again the leptomonas form, includes the parasites of leishmaniasis. Here, however, there is a vertebrate host in which the leishmaniform parasite is most usually seen, but also occasionally the leptomonas, as I have shown (1915). The latter is generally encountered only in cultures in the test-tube or in certain invertebrates which have ingested the leishmania forms along with a quantity of blood. There is undoubtedly an invertebrate host of this flagellate and in it the infection would be expected to resemble a true leptomonas of an invertebrate. In order to distinguish these flagellates which have a vertebrate as well as an invertebrate host from the purely insect form—the true *Leptomonas*—the generic name *Leishmania* can be employed.

A third group of flagellates attains a still higher stage of development and the individual flagellate is known as a crithidia. In it the kinetoplast is close to, but still in front of, the nucleus, and there is a short undulating membrane running from just in front of the kinetoplast to the anterior end of the flagellate. Along the edge of this runs the flagellum, to become free at the anterior extremity of the flagellate. The crithidia live in the intestine of invertebrates and in the posterior part of the intestine there are produced small leishmania forms, as in the leptomonas, and they are responsible for the spread of infection from host to host. There is again only a single host—an invertebrate—and such forms may be considered under the generic name *Crithidia*. We know of no flagellate of this type which has both a vertebrate and an invertebrate host corresponding to the *Leishmania*.

A fourth group shows a still higher development in that the true trypanosome structure is attained. The kinetoplast is further back at the posterior

end of the body and there is a long undulating membrane along which the flagellum runs, to become free at the anterior extremity of the flagellate. In the insect's intestine these forms show not only the trypanosome type but also intermediate ones—crithidia and leptomonas—and finally the leishmania forms. In flagellates of this group there is again only a single invertebrate host and the infection is spread from insect to insect by the leishmania forms as in *Leptomonas* and *Crithidia*. A flagellate of this group is *Herpetomonas muscae domesticae* of the common house-fly and it is convenient to distinguish these forms by the generic name *Herpetomonas*.

In a fifth group there are the same forms as in the preceding group, but in place of the single invertebrate host there is a vertebrate one as well. This is the group of true trypanosomes which have the generic name *Trypanosoma*.

This scheme of classification may have many points against it, but at any rate it is convenient and is as far as we can go in our present state of knowledge. To subdivide these groups into different genera simply leads to further confusion. All we can do is to wait for further data, and such an attempt as that made by the late Dr Albert Chalmers (1918) to split up the genus *Trypanosoma* into his extraordinary series of new genera only tends to bring confusion into an already difficult subject.






The scheme I have outlined above has the merit of simplicity and it can be arranged in tabular form in the following manner. The names in the right-hand column (Text-fig. 1) are merely descriptive and should be used in an adjectival sense. They may be employed for any particular stage which may appear in the development. Those in the left-hand column are generic titles and, as such, should be written in italics and with a capital. We can speak, for instance, of the leptomonas stage of *Leishmania tropica*, the crithidia stage of *Trypanosoma lewisi*, or the leishmania stage of *Herpetomonas muscae domesticae*.

There is one group of flagellates which do not enter into the above scheme. I refer to the curious leptomonas form first described by Lafont from the latex of Euphorbias as *Leptomonas davidi*. The work of Lafont, Bouet and Roubaud, and more recently of França (1919), has demonstrated that this flagellate undergoes a cycle in a hemipteron and is transmitted by it to healthy plants. Thus we have a flagellate of the leptomonas type which has both an insect and a plant host. Evidently it cannot be included in any of the genera defined above. For these flagellates we may employ the name *Phytomonas*, first suggested by Donovan (*Lancet*, 1909), the type species being *Phytomonas davidi* (Lafont, 1909).

If the flagellates of this most confusing group were named according to the scheme outlined above we should at any rate understand from their names something of their life history and structure.

The question now arises into what group we are to place the flagellates of the gecko, the anolis and the chamaeleon. I think we are safe in assuming that there must be invertebrate hosts. The two former would undoubtedly

fall into the group leishmania, according to the definition given above, and would receive the provisional names of *Leishmania tarantolae* and *Leishmania Henrici*. If it should subsequently be found that the flagellate of the gecko is in reality identical with that causing Oriental sore then it would of course become *Leishmania tropica*, but proof of this is at present wanting. The flagellate of the chamaeleon occupies an intermediate position. It undoubtedly has an insect host but, unlike the parasite of the gecko, it is not a tissue parasite and as far as my investigations go it appears to be confined to the

GENERIC TITLES		FLAGELLATE TYPES	
TRYPANOSOMA	HERPETOMONAS		IN VERTEBRATE HOST
		TRYPANOSOME	
			IN
		TRYPANOSOME	
			INVERTEBRATE
		CRITHIDIA	
	LEISHMANIA		HOST
		LEPTOMONAS	
			IN VERTEBRATE HOST
		LEISHMANIA	

Text-fig. 1. Diagram of classification of the trypanosomes and allied flagellates.

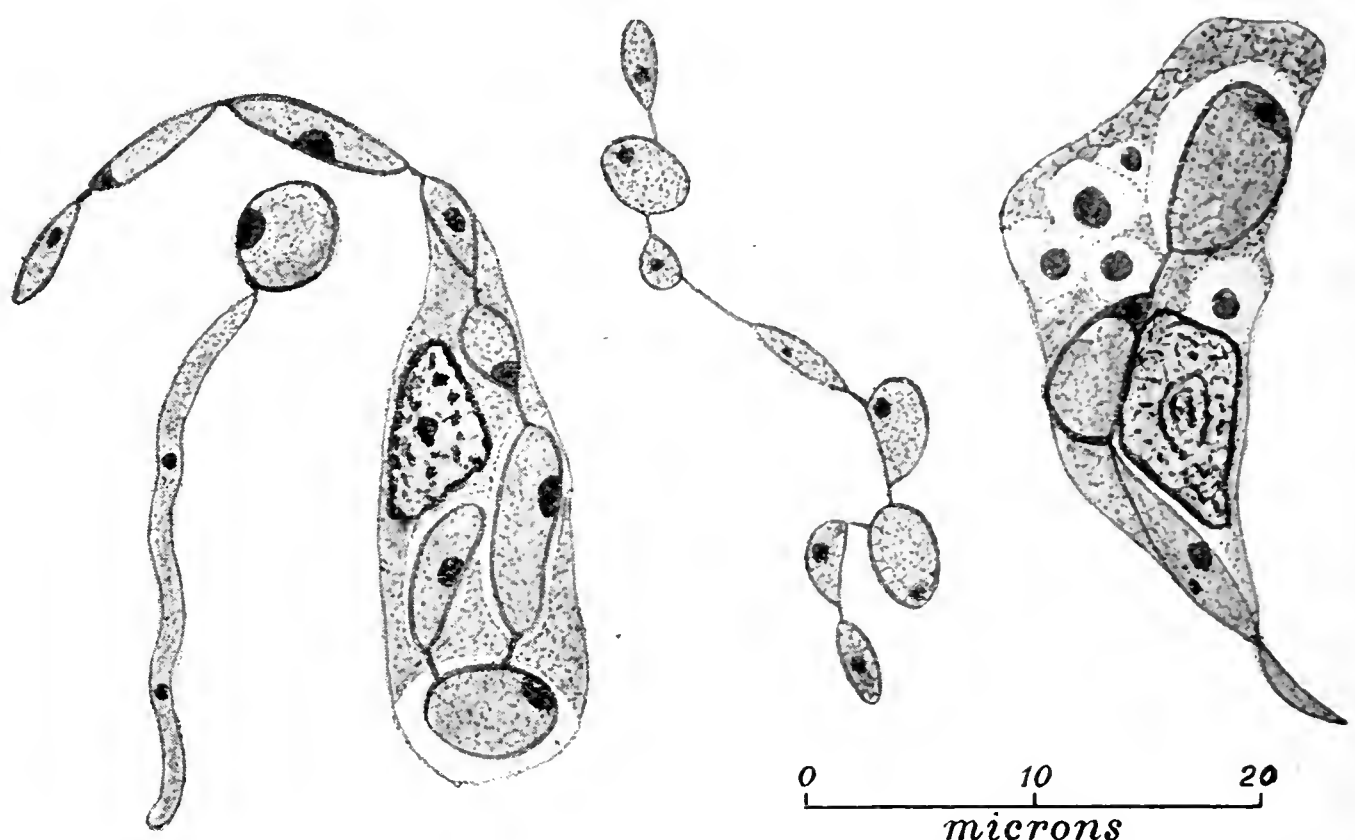
intestine and cloaca. However, it comes within the definition as having both hosts and it will therefore become *Leishmania chamaeleonis*. It seems better to place it in the genus *Leishmania* than in *Leptomonas*, for the latter would imply that it was a purely insect, or at least invertebrate, flagellate.

The intestinal habitat is of interest as it is an infection probably acquired from some insect and indicates a stage by which such an insect flagellate may eventually become a tissue parasite. It occupies an intermediate position between the purely insect flagellate and the vertebrate ones such as that of

the gecko, and of Oriental sore and kala-azar. Moreover it suggests the possibility of an intestinal mode of infection in the latter disease. In the case of *L. Henrici* it appears as if the intestinal infection, still persisting in the lizard, has already given rise to a blood and tissue infection.

Note on a cell-invading fungus.

In some of the chamaeleons examined it was noted that a fungus (Text-fig. 2) was present, and in smears and sections it was found that there was a definite invasion of the cells of the cloaca. The fungus occurs in the form of ovoid bodies embedded in the cytoplasm of the cells. Reproduction takes place by budding till the whole cell is invaded. In this process the nucleus becomes pushed aside and distorted and the cell eventually degenerates. Buds are



Text-fig. 2. The fungus which was found invading the cells of the cloaca of *Chamaeleon vulgaris*. C. M. W. del.

also formed, protruding into the cavity of the cloaca and when this has occurred growth into the lumen takes place with the formation of more elongate elements and finally filaments. No culture of this organism was attempted and nothing more of its life-history is known. The general characters are depicted in the drawing shown in Text-fig. 2.

2. *LACERTA AGILIS* AND *AGAMA STELLIO*.

The protozoal organisms seen in the gut of these two lizards resembled one another and I have no reason to suppose that the forms common to these two lizards belong to different species. Those common to the two are *Bodo lacertae*, Grassi, or, as Alexeieff (1912) has renamed it, *Prowazekella lacertae*, *Chilomastix* sp., and *Entamoeba* sp. In addition to these three organisms, in

Agama stellio was also found a trichomonas and a trichomastix. These are probably identical with *Trichomonas lacertae*, Prowazek, and *Trichomastix lacertae*, Bütschli. It is possible that if a number of *Lacerta agilis* had been examined they would have been found in them also. The Entamoeba may possibly be identical with one seen by Dobell (1914) in the gut of *Lacerta muralis*.

Prowazekella lacertae Grassi.

This flagellate has been the subject of some controversy on account of its association in the gut of the lizard with a blastocystis, or structures which have a striking resemblance to this. Prowazek (1904) claimed to have demonstrated that the blastocystis was in reality the autogamy cyst of the flagellate. It is very improbable that any autogamy takes place in the life-history of the flagellate, but nevertheless it is undoubtedly true that the flagellate encysts and that the cysts produced have frequently been spoken of as blastocystis. Chatton (1917) describes the blastocystis as a stage in the developmental cycle of the flagellate and finds that under certain conditions not well understood the large blastocystis resulting from growth of the smaller forms produces large numbers of flagellates. He supposes, and probably correctly, that a conjugation between the flagellates takes place in association with the encystment.

Description of flagellate.

The flagellate (Pl. XX, Fig. 3) is in its adult stage an elongate, flattened organism with two tapering flagella, one of which is directed forwards and is several times the length of the body, and the other, a thinner and shorter one, directed backwards. There is a single anterior nucleus, on the anterior surface of the membrane of which lies a granule from which the flagella arise.

The body of the flagellate, as already mentioned, is flattened like a blade of grass, but not perhaps to the same extent, and sometimes, as noted by Prowazek (1904), the edges are folded or the body may be twisted on itself, but as usually seen it is simple in structure with the posterior end pointed and tapering to a varying extent. The anterior end is also pointed but less acutely than the posterior end. The cytoplasm is vacuolated and may contain granules. Near the anterior end lies the spherical nucleus, which is closely surrounded by a number of deeply staining bodies. In iron haematoxylin preparations these tend to obscure the nucleus unless the differentiation is carried far enough. The nucleus itself is a spherical body consisting of a nuclear membrane and a centrally placed karyosome. Fine granules may also be seen within the membrane, either on its inner surface or between it and the karyosome. On the anterior surface of the membrane is the granule from which the rhizoplast arises. This granule does not retain the stain as intensely as the karyosome of the nucleus nor the bodies which surround the nucleus. In some cases it appears that the granule may be double but it is difficult to make out whether this is merely an early stage of division or not. From the

granule there runs forward a fine rhizoplast. The deeply staining bodies which surround the nucleus vary considerably in number and arrangement. Sometimes they are closely applied to the nuclear membrane while at others they are separated from it by a distinct interval. There may be many fine granules or a few larger masses. They may surround the nucleus or be limited to one side only or be entirely behind it. There may be only two bodies, one on each side of the nucleus, and of such a shape that they are swollen posteriorly and tapering anteriorly, with their anterior extremities nearer one another than the posterior ones. Prowazek (1904) notes these bodies and regards them as composed of chromatin material derived from the nucleus. I can find no evidence that these bodies arise from the nucleus. In those cases where they appear to be within the membrane careful observation shows that they are merely overlying it. Prowazek (1904) figures forms in which these bodies are on the inner surface of the membrane but in all the forms I have seen they are outside it even if lying against it. Prowazek's idea that they are chromidial in nature was first contested by Dobell (1908), who wrote: "I think sufficient has been said to show that autogamy and chromidia are as yet unproven in the case of *Bodo*." They can best be spoken of as parabasal bodies similar to those which are associated with the origin of flagella in other flagellates.

The rhizoplast arising from the blepharoplast or centrosome on the nuclear membrane passes forwards and is continued into the two flagella. At the extreme anterior end of the organism at the point where the flagella arise from the rhizoplast is occasionally seen a granule or thickening but I have been unable to make out any structure in this (Pl. XX, Fig. 3, *a, c, g*). Prowazek (1904) figures the rhizoplast as terminating near the anterior end of the body in a sort of cone, beyond which is a second granule from which the flagella actually arise. If this structure is really present and there is a break in the rhizoplast then the flagellar origin is of a very specialized type. Is it not possible that this appearance is due to some peculiar plasticity of the anterior end of the body, which by retraction at the point of exit of the flagella gives rise to the cone-like appearance? At any rate in my preparations many of the flagellates seem to have a rhizoplast continued directly from the blepharoplast into the flagella. I have been unable to trace any connexion between the blepharoplast and a granule within the karyosome of the nucleus such as Prowazek describes.

The anteriorly directed flagellum is often at least five times the length of the body of the elongate flagellates and in some of the smaller and ovoid forms it is even longer in proportion. The posteriorly directed flagellum reaches as much as three times the length of the body in the long forms. It is very much finer than the anteriorly directed flagellum and in some individuals it appears to be attached to the body for a short distance (Pl. XX, Fig. 3, *a, c, e*), an attachment which may have to do with maintaining its direction. In many of the flagellates, however, there is no such attachment.

Multiplication.

The flagellate multiplies in two ways, either by simple division or cyst formation. I have not been able to trace the division in much detail, as dividing flagellates were not numerous in my preparations. However, evident dividing forms were seen in which two nuclei were present, each with its blepharoplast and rhizoplast passing into two flagella. The parabasal bodies were divided between the two nuclei so that in nuclear division those of the original nucleus are evidently divided into two more or less equal groups, as occurs in nuclear division within the cyst.

Encystment takes place in the hinder part of the gut of the lizard and is preceded by a change in shape of the organism. It becomes an ovoid body and apparently two of these become encysted together (Pl. XX, Fig. 3, *n*). A feature of the encystment is that clumps occur, the individuals of which are all in approximately the same stage of development. The clump appears to be held together by an adhesive material in which various bacteria and other intestinal debris are included.

The first stage is the formation of the ovoid body, which loses its flagella. Two of these come together and a cyst wall is formed round the pair (Fig. 3, *j* and *o*). The cyst wall is evidently of a gelatinous nature, for bacteria and other debris adhere to it. Within the cyst the two organisms fuse, as noted by Prowazek (1904), and this, I believe, is followed by fusion of the nuclei (Fig. 3, *p-s*). I can see no evidence of an autogamy as described by him.

The next stage is the appearance of a vacuole in the cytoplasm of the zygote and this gradually increases in size, evidently by absorption of fluid through the cyst wall, till the cyst becomes many times its original bulk. Concurrently with this vacuolation nuclear division takes place (Fig. 3, *t-y*). The original nucleus of the zygote has the same structure as that of the flagellates, being spherical with a nuclear membrane and central karyosome (Fig. 3, *r* and *s*). The parabasal bodies lie around the nucleus. The exact similarity between the nuclei of the cyst and the flagellates makes it practically certain that they are in reality flagellate cysts, quite apart from the stages of encystment where every step can be followed. In division the karyosome divides and the two parts separate, while they remain connected by a fine fibre (Fig. 3, *u-w*). The nuclear membrane elongates at the same time, and finally two nuclei are formed by constriction at the middle. The parabasal bodies remain at the equator of the elongating nucleus for some time and then they are divided into two groups which pass to the daughter nuclei. These bodies are of various sizes and they do not appear to form anything in the nature of chromosomes. I cannot say if any actual division of each separate mass takes place but each of the resulting groups of daughter parabasal bodies contains more or less an equal amount of material. The nuclei continue to divide in the same manner till as many as 32 are formed within the now very enlarged cyst (Fig. 3, *m, l, i*). The majority of the cysts are spherical but some are

elongate or even dumb-bell shaped. The growth of the cyst is remarkable but a similar growth takes place during the development of the oöcyst of the malarial parasite in the stomach of the mosquito. It is evident that the cysts are not very resistant bodies, but that they are destined to pass out of the body is supported by their occurrence in greatest number at the hinder end of the intestine.

The fully formed cyst contains comparatively little cytoplasm, which is grouped around the nuclei on the inner surface of the cyst wall. The bulk of the cyst contains liquid, through which strands of a coarse network of more refractile material run and which connect with the nuclear areas. Even though not possessed of very tough or impermeable walls it is possible that these cysts would take a considerable time to dry completely on account of their enormous fluid content. The development of the cyst beyond the 32 nuclear stage I have not been able to follow but Prowazek (1904) and later Chatton (1917) have seen these large cysts give rise to numbers of flagellates.

The description I have given depends entirely on stained films as prolonged observations on the living cysts were not made. That two individuals encyst together seems undoubted but if one wished to be hypercritical one could suppose that the two associated individuals either before or after encystment were the results of division and do not represent conjugation. My interpretation of the appearances seems the more probable one and is in conformity with Chatton's own observations. It must, however, be admitted that an absolute proof of the process has not been obtained by me. Further observations on this parasite might yield some interesting facts in connection with the conjugation process of flagellates and the origin of the parabasal bodies and centrosome.

Blastocystis.

As already pointed out, many of these flagellate cysts bear a striking resemblance to blastocystis and as a matter of fact I have been accustomed to regard them as such. In the lizards I have examined, all the so-called blastocystis have the very characteristic nucleus with the surrounding parabasal bodies, so there can be no doubt that they are stages in the development of the flagellate cysts. Whether side by side with the true flagellate cysts there exists a "Blastocystis" of vegetable nature I cannot say at present, but it cannot be doubted that *Prowazekella lacertae* encysts in the gut of the lizard and that the cysts have been frequently styled "blastocystis."

What then is the blastocystis which occurs so commonly in the human intestine and that of other animals? Prowazek (1911) maintained that they were cysts of *Trichomonas* but there is no evidence to support this view. Swellengrebel (1917) has suggested that they are degenerate forms of various intestinal protozoa, while Jepps and Dobell (1918) have noted that certain degenerate forms of *Dientamoeba fragilis* resemble dead blastocystis. I myself have, for want of evidence to the contrary, always regarded them as of a vegetable nature and this may be the case in spite of the resemblance to

the cysts of *Prowazekella lacertae*. For a similar reason I regarded the I-cysts as being probably vegetable organisms, but they are now known to be cysts of *Iodamoeba williamsi*. I had also noted (1910) that degenerating *Chilomastix mesnili* could assume appearances closely resembling blastocystis. Swellengrebel's (1917) conclusion is that blastocystis "is not the name of a zoological genus but of a peculiar form of degeneration to which representatives of different genera of intestinal protozoa may be liable." On the other hand future investigations may show that blastocystis is derived from amoebae and it must be admitted that the large binucleate cysts of *Entamoeba coli* with the large vacuole occupying almost the entire cyst bears some resemblance to binucleate forms of "blastocystis." Frequently in stained preparations containing small entamoebae, such as *Endolimax nana*, and blastocystis, it is possible to trace what might be regarded as a complete series of connecting links between a typical amoeba and a typical blastocystis and one is constantly tempted to adopt the view that the series traceable is a real one. Macfie (1915) regarded certain blastocystis associated with an entamoeba in the monkey *Cercopithecus petaurista* as cysts of the entamoeba. His proof of this, however, appears to be wanting and furthermore we know that the entamoebae of the monkey produce the typical entamoebic cysts with four or eight nuclei.

Alexeieff (1911) says that at one time he regarded the blastocystis of the lizard as derived from the lizard flagellate but that later observations have altered his opinion and that he has come to look upon blastocystis as a purely vegetable organism. He was largely influenced in this by the character of the development of a yeast (*Schizosaccharomyces octosporus*), and has suggested the name *Blastocystis enterocola* for the intestinal blastocystis.

Dobell (1908) in criticizing Prowazek's work on the autogamy cysts of *Bodo* compares them with very similar cysts he had seen in the gut of the frog and which he proved by germination to be of a vegetable nature. It appears to me that Prowazek's cysts, though not autogamy cysts as he describes them, are at any rate true cysts of the flagellate. At least this can be stated of some of those he figures.

It is evident therefore that there is a difference of opinion as to the true nature of blastocystis and we must await further information. It seems possible that under the name blastocystis three distinct structures have been confused: the true protozoal cysts like those of the lizard flagellates, vegetable organisms like the cysts Dobell studied in the frog or which Alexeieff saw in the case of his yeast *Schizosaccharomyces octosporus*, and thirdly, degenerate intestinal protozoa or even tissue cells, some of those in the human intestine belonging to the second group and others to the third.

Trichomonas lacertae Prowazek, and *Trichomastix lacertae* Bütschli.

I have nothing to add to the description of these two organisms as given by Prowazek (1904). I have seen none of the conjugation forms described by him. The flagellates are shown in Pl. XX, Fig. 2, *h* and *i*.

Chilomastix sp.

This is a small organism of the usual structure and in size corresponds with the two flagellates just mentioned. Its general appearance is shown in Pl. XX, Fig. 2, *g*.

Entamoeba sp.

The entamoeba is a large organism in its fully grown form (Pl. XX, Fig. 2, *a*). Smaller forms also occur and the general character of the organism is shown in Fig. 2, *a-f*. It will be noted that it bears a striking resemblance to *Entamoeba coli* and, like it, it feeds upon most of the contents of the lizard's intestine, frequently ingesting the flagellates or their cysts (Fig. 2, *b*). It produces an eight-nuclear cyst which again is not distinguishable from that of the human *Entamoeba coli* either in size or characters.

Of the lizard hosts mentioned above, judging from the contents of their intestines *Chamaeleon vulgaris* is entirely an insect feeder. This is true to a large extent of *Lacerta agilis*, though in it some vegetable matter is also present. *Agama stellio* feeds on insects but also largely on vegetable matter, as large pieces of leaves of plants and grass are to be found in the stomach. It is not surprising therefore that the two last named can infect themselves from vegetable matter contaminated by other infected lizards, while the chamaeleon only harboured the flagellate which it had probably acquired from some insect, which had in its turn taken up the flagellate from the faeces or rather cloacal mucus of the animal.

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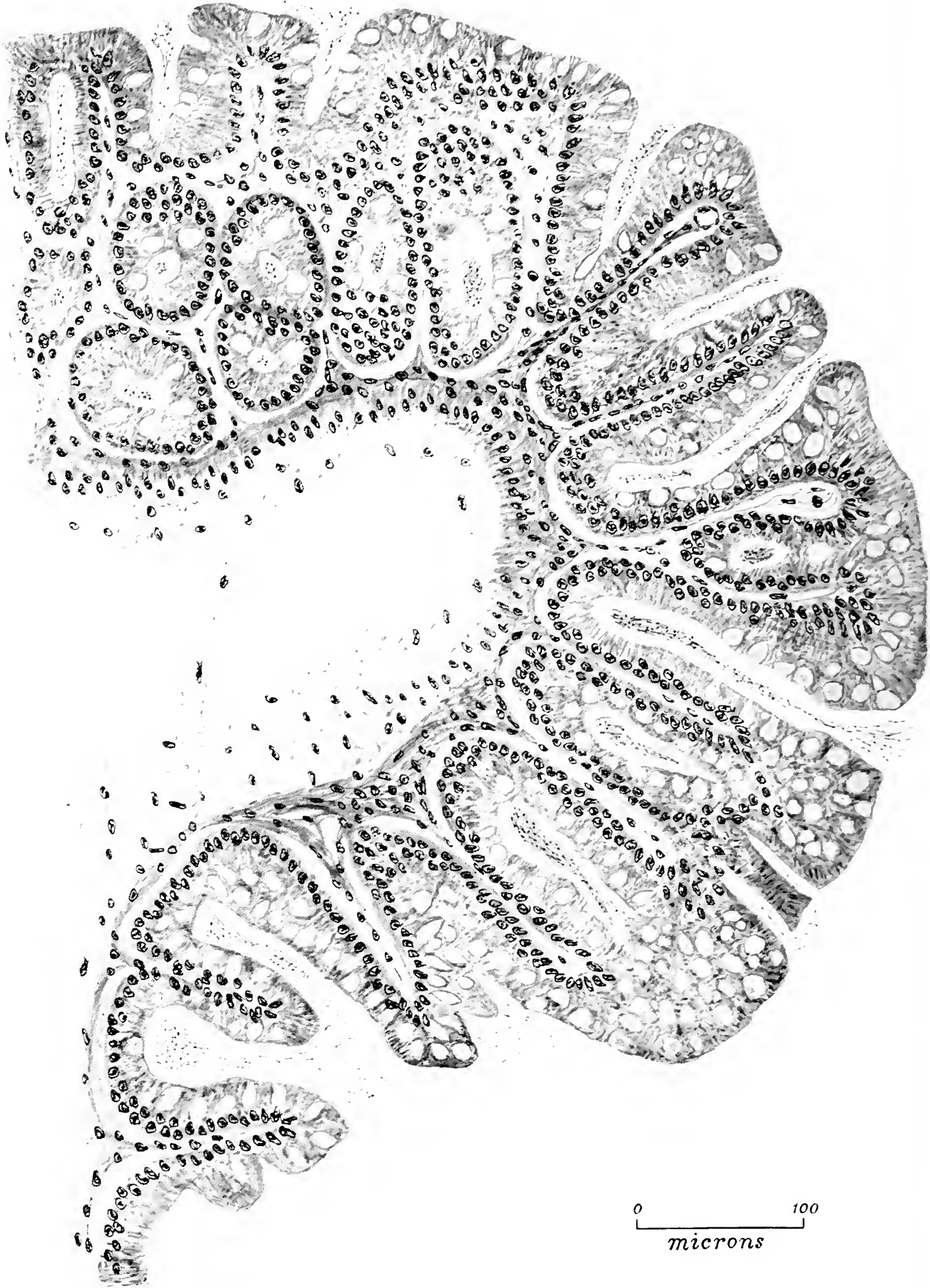


Fig. 1. Section through part of the wall of the cloaca of *Chamaeleon vulgaris* to show the lumen of the glands filled with clusters of *Leishmania chamaeleonis*. (C. M. W. del.)

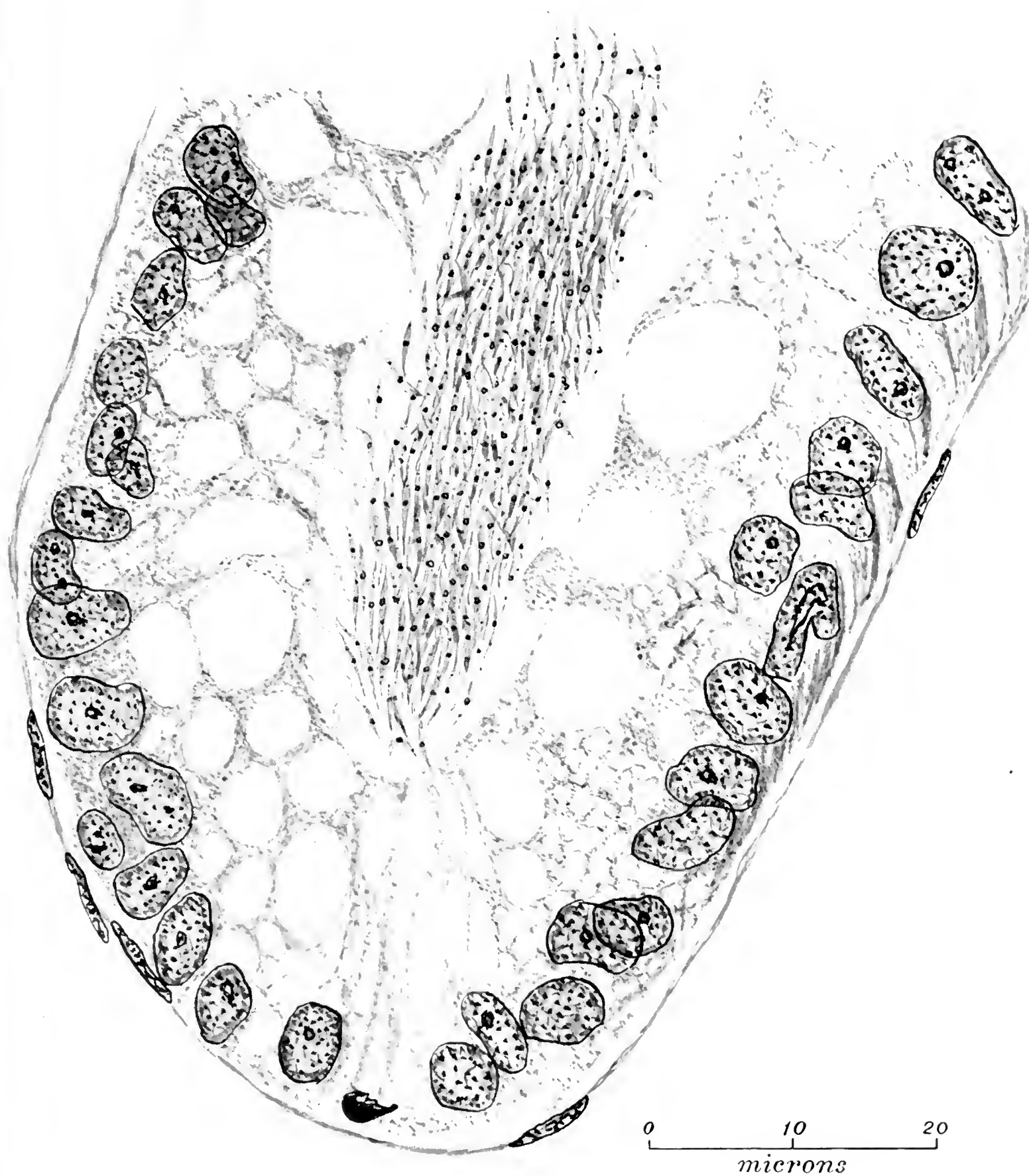


Fig. 2. A single gland in the same section as shown in Fig. 1 drawn to a larger scale. There appeared to be no invasion of the cells by the flagellates. (C. M. W. del.)

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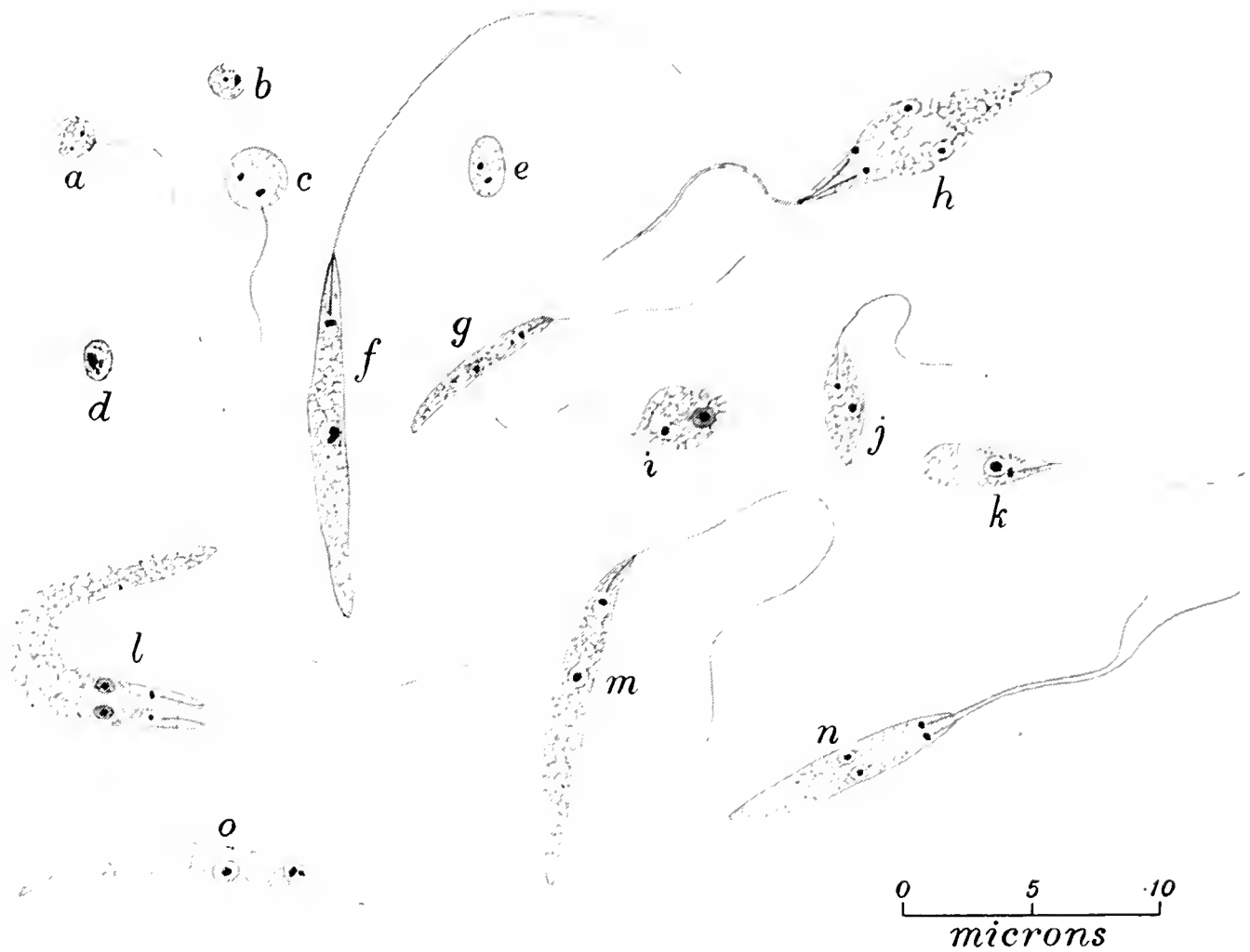


Fig. 1. Various types of *Leishmania chamaeleonis* found in *Chamaeleon vulgaris*. Every intermediate shape and size between the long forms (*f*, *l*, *m*, *n*, *o*) and the minute ones (*a*, *b*, *c*) are to be found. A possibly encysted form is shown at *d*. (C. M. W. del.)

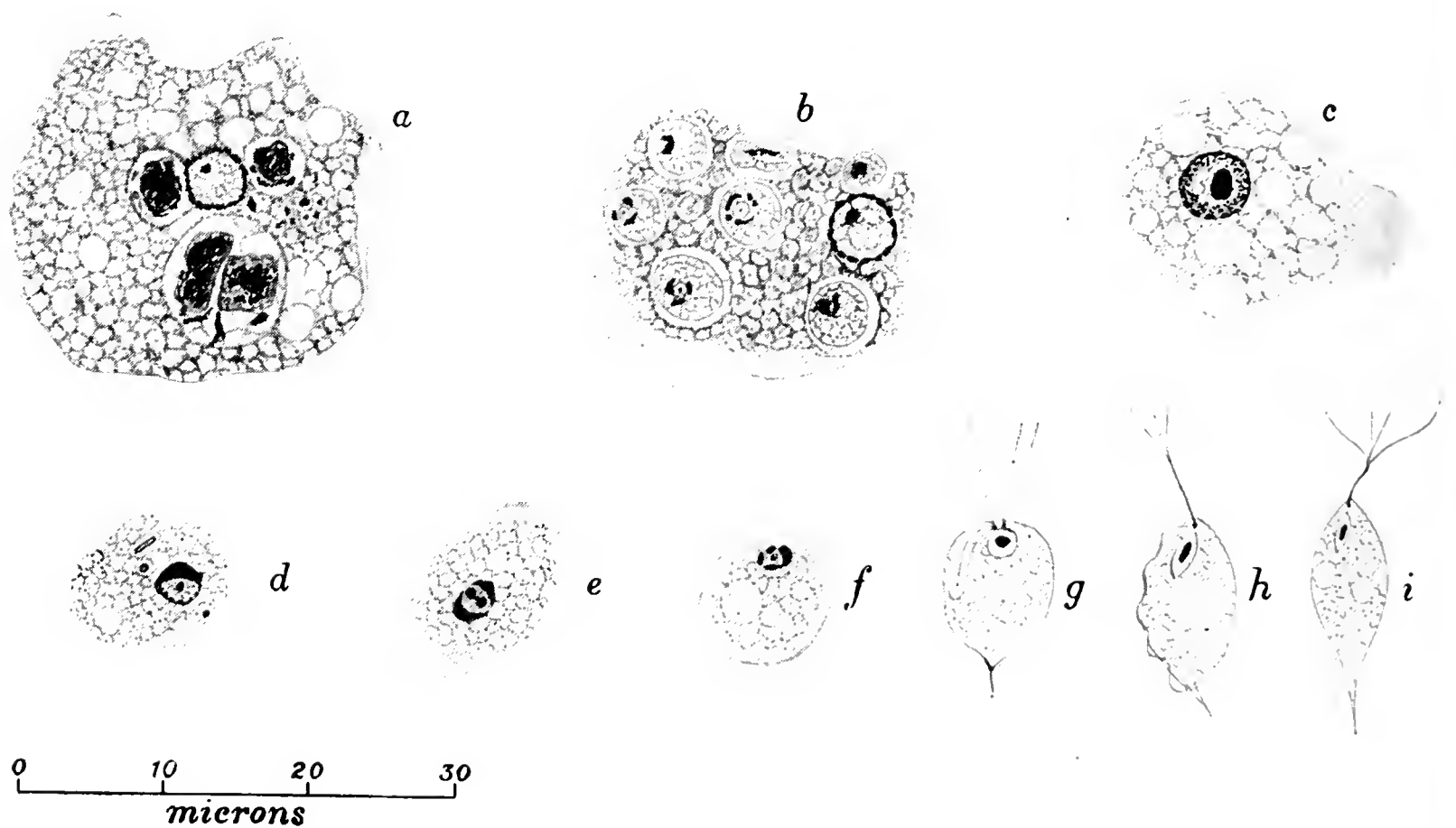


Fig. 2. Protozoa found in *Lacerta agilis* and *Agama stellio*. The entamoeba which produces cysts exactly like those of *Eutamoeba coli* is shown at *a*—*f*; *Chilomastix* sp. at *g*; *Trichomonas lacertae* at *h*; *Trichomastix lacertae* at *i*. (C. M. W. del.)

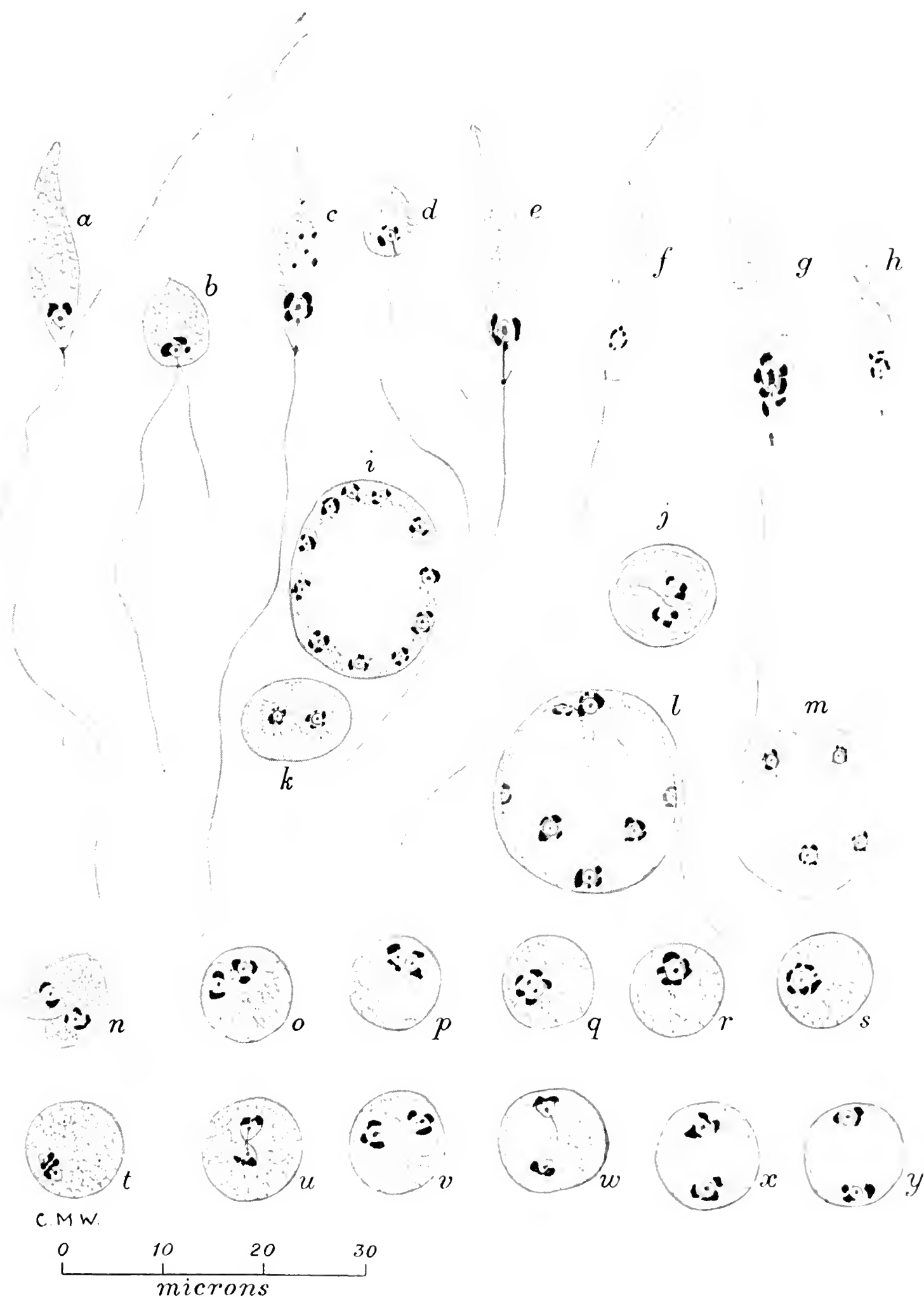


Fig. 3. *Prowazekella lacertae*. The forms figured at *a*, *c* and *e* show the appearance of the attachment of the "trailing" flagellum. In others both flagella appear to be quite free. The series *n* to *s* shows what is probably the conjugation and encystment, while *t* to *y* show the first nuclear division of the zygote and the formation of the vacuole. At *j* and *k* are two cysts corresponding to that at *o*. Later stages of the cyst development are shown at *m*, *l* and *i* in which nuclear multiplication to the 32 nuclear stage is reached. At all stages the parabasal bodies are seen surrounding the nucleus and they are roughly divided into two groups at nuclear division both of the free flagellates and the encysted forms. (C. M. W. del.)



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LEISHMANIA, HERPETOMONAS, AND CRITHIDIA IN FLEAS.

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(With Plates XXI and XXII.)

INTRODUCTION.

MOST of the observations recorded in this paper would have been published in 1915, if my duties as a doctor and as an officer had not called me to the Army.

Following on my experimental researches (1910–1915), on the transmission of visceral leishmaniasis by means of fleas (*Ctenocephalus canis* and *Pulex irritans*), a very interesting problem arose which still rivets the attention of students of this subject.

In the intestine of various insects and of fleas in particular, certain Protozoa of the herpetomonad and crithidial types have been observed. The morphological, biological and experimental study of these Protozoa in relation to *Leishmania* is particularly interesting.

Patton maintained that this parasite is a true *Herpetomonas*, and called "herpetomoniasis" those infections which we now call "leishmaniasis." Minchin and Wenyon, on the other hand, observed that although *Leishmania* in some stages presents morphological characteristics almost identical with *Herpetomonas* it is distinguished by the fact that *Herpetomonas* is only found in insects, in whose intestines the different species undergo their complete developmental cycle (flagellate and non-flagellate), while *Leishmania* completes its cycle in two hosts, one of which is a vertebrate and the other an invertebrate. In the vertebrate host *Leishmania* produces a disease with characteristic symptoms—"Leishmaniasis."

BIOLOGY AND MORPHOLOGY OF *LEISHMANIA*.

The biological and morphological characteristics of the parasite must be studied, (a) in the vertebrate host; (b) in artificial cultures; (c) in the transmitting host.

(a) *Leishmania* in the vertebrate host is non-flagellate, it appears either oval (Plate XXI, fig. 1), or round, with a diameter varying from 2 to 4 μ ; it may also be pear-shaped (Plate XXI, fig. 3) when it may attain a length of 7 μ .

In the parasite we can distinguish two masses of chromatin, the larger is called the nucleus, or trophonucleus, the smaller is called the blepharoplast, or kinetonucleus. Novy has also described a small well defined organ, the rhizoblast. The plasma appears finely granulated and in the large forms shows vacuoles.

The nucleus is rounded and often appears as a compact mass (Plate XXI, fig. 1); occasionally it seems to consist of small grains of chromatin; at times it shows a minute body more deeply coloured, placed centrally or eccentrically, which recalls the karyosome (in *sensu lato*); very rarely the nucleus is absent.

The blepharoplast is rod-shaped (Plate XXI, fig. 1) or pointed (Plate XXI, fig. 2), almost always detached from, but at times attached to the nucleus (Plate XXI, fig. 2). Visentini, who has carefully studied the morphology of *Leishmania* in the vertebrate host, thinks that this attachment of the blepharoplast to the nucleus indicates the nuclear origin of the blepharoplast; sometimes the blepharoplast may be absent (Plate XXI, figs. 4, 5).

In parasites derived from haematopoietic organs, the rhizoblast occurs as a minute body or rod placed between the kinetonucleus and the peripheral zone of the parasite. In canine leishmaniasis, smears from such organs reveal the presence of granular forms which I believe to be connected with *Leishmania*, but I cannot say definitely whether they represent a phase of the developmental cycle and are comparable with the granular forms of trypanosomes, or whether they represent degenerative forms of the parasite.

Reproduction. According to all authorities, *Leishmania* reproduces itself by simple longitudinal division; division of the nucleus and of the blepharoplast is first observed (Plate XXI, figs. 5, 6); then the plasma divides. This form of agamic reproduction leads to the formation of characteristic bodies consisting of several parasites enclosed in a common protoplasm; Ross suspected a sporulation phase; Nicolle on the other hand, considered them to be parasites enclosed in the plasma of the leucocytes whose nucleus might have become detached in the process of preparing the smear.

Besides the reproduction by simple longitudinal division, from which two individuals morphologically indistinguishable are produced (equal division), I have found that sometimes the longitudinal division gives origin to two individuals which can be morphologically distinguished (unequal division); I call the equal division "homomorphous" and the unequal division "heteromorphous."

In heteromorphous division the blepharoplast takes no part whatever in the process of division, which occurs only in the nucleus (Plate XXI, fig. 7).

Visentini described, without giving any explanation, the existence of parasites consisting of plasma enclosing a nucleus but no blepharoplast (Plate XXI, fig. 4). I have decided that such forms result from an unequal division wherein no division of the blepharoplast occurs, the nucleus dividing into two equal similar nuclei, the plasma also dividing immediately. Two parasites are thus

produced, one of which presents a nucleus in which the karyosome is not visible, but which contains the maternal blepharoplast, while the other parasite contains no blepharoplast but shows a nucleus with a karyosome (Plate XXI, fig. 7). This explains the appearance already minutely described by Visentini, and leads us to believe in the nuclear origin of the blepharoplast.

The heteromorphous division above described is probably of significance in the life cycle of *Leishmania*. The development of a parasite which at first is apparently without a blepharoplast is an important phenomenon; I think that some forms of the parasite may represent resistant forms which are of use in the maintenance of the species and which are produced under special conditions. I base this deduction on the fact that I have most frequently met with these forms in the haematopoietic organs of dogs naturally infected with leishmaniasis which was running a chronic course.

(b) *Leishmania in artificial cultures*. Rogers, who was the first to cultivate *Leishmania donovani* did so by adding citric acid or citrate of soda in the proportion of 10 per cent. to the spleen or liver juice of individuals affected with leishmaniasis (Indian virus); his experiments being confirmed by Leishman and Statham whilst Longo subsequently cultivated the Mediterranean *Leishmania*. Nicolle, in 1907, cultivated the parasite in the Novy-McNeal medium, and, in 1908, in the Novy-McNeal-Nicolle medium.

Row, in 1912, obtained the cultural development of *Leishmania donovani* (Indian virus) in a haemoglobinised saline solution and Visentini used this medium for cultivating *Leishmania infantum*. *Leishmania* also develops in Bordet and Gengou's medium, in human blood agar and in Jemma's ascitic-agar medium. The temperature is of the highest importance in the cultural development of these parasites; they develop at a temperature of between 18° C. and 25° C., with the best results at 20° C. to 22° C.

After inoculation, from four to five days, on an average, are required before flagellate forms of *Leishmania* appear in the culture fluid. This development may however be completed in a shorter period. Visentini and I once saw a few flagellates appear 48 hours after cultures on N.N.N. medium had been inoculated with the splenic juice of a dog intensely infected with leishmaniasis. After several days non-flagellated forms reappeared and we regard these post-flagellate forms as representing resistant stages.

During the first hours after inoculation the young non-flagellate parasites resemble the forms seen in the blood (peripheral and from haemopoietic organs); gradually they become pear-shaped (Plate XXI, fig. 8) and the flagellum develops (Plate XXI, fig. 9). Such flagellate piriform parasites usually measure 8–12 μ in length by 2–3 μ in breadth. Besides the pear-shaped parasites there occur some elongated forms (Plate XXI, figs. 10, 11, 12), with a pointed front end from which the flagellum projects. *Leishmania* in cultures reproduces itself by simple longitudinal division and this leads to the formation of slender elongated parasites (Plate XXI, figs. 13, 14, 15).

After some days, there appear flagellate parasites of large size which may

measure up to $26\mu \times 5\mu$. The appearance of these large forms is evidently due to the artificial conditions prevailing in the culture, and, since many of them show vacuolated plasma, they may well represent degenerative forms.

In addition, elongated and spherical forms of rather large size and with granules scattered in the plasma are to be noted. These forms have been regarded as sexual forms; or (Rogers, Marzinowski) as conjugation forms. I have made repeated and prolonged observations in this regard, using a thermostat at 22°C ., but I have never observed conjugation to take place.

The morphological study of cultural *Leishmania* both of infantile and canine origin has been carefully followed by Visentini at the Lister Institute. I shall refer to the principal deductions of this writer, dealing chiefly with the morphological identity between the evolutive forms of *Leishmania* in fleas and the forms encountered in cultures.

(c) *Leishmania* in the transmitting host (*Ctenocephalus canis*, *Pulex irritans*). Patton, describing the life cycle of the flagellate Protozoa of insects (*Herpetomonas* and *Crithidia*), has distinguished three stages—the pre-flagellate, the flagellate, and the post-flagellate. These stages are also to be noted in the cycle of *Leishmania* in fleas. The pre-flagellate stage is found in the flea's mid-gut; the flagellate stage in the hind-gut; and the post-flagellate stage in the rectum and faeces; these three stages correspond morphologically to those of *Leishmania* in cultures.

In the flea's mid-gut, the parasites show the same characteristics which they present in the peripheral blood and in the haematopoietic organs of infected vertebrates; when fixed and coloured they appear rounded, oval, or pear-shaped (Plate XXI, figs. 16–19), they measure on an average $2\text{--}4\mu$ in diameter. The protoplasm assumes a delicate pale blue tint; the nucleus of a reddish violet colour is almost always compact, sometimes granular; the blepharoplast is sometimes absent, as has been noted in parasites from the haematopoietic organs; when it is present it may be rounded or rod-shaped and of the same, or a deeper tint than the nucleus.

Leishmania in this pre-flagellate stage divides longitudinally; first the nuclei and then the plasma. In this manner forms are produced indistinguishable morphologically from those in the haematopoietic organs (Plate XXI, figs. 20, 21, 22).

In some stained preparations however, I have seen certain parasites (Plate XXI, fig. 23), which show two nuclei and a blepharoplast. I have carried out numerous experiments to ascertain whether these might represent sexual forms, but with no success.

In fleas experimentally infected and kept at a temperature of 22°C ., after 36 hours from infection I have observed flagellated forms in the intestine, these are morphologically identical with the pear-shaped or elongated cultural forms which have been already described.

In fact, these flagellate, pear-shaped parasites in fleas have almost the same measurements and appearance as the cultural forms being $7\text{--}9\mu$ in

length, exclusive of the flagellum, and $2-4\mu$ in breadth (Plate XXI, figs. 24, 25, 26). The blepharoplast is situated in front of the nucleus, to which it is more or less contiguous (Plate XXI, figs. 24, 26); in parasites which are nearing the post-flagellate stage it is situated laterally to the nucleus (Plate XXI, fig. 25). The flagellum may attain a length of $20-22\mu$; it may develop from a basal granule (Plate XXI, figs. 25, 26, 27), or directly from the blepharoplast (Plate XXI, figs. 28, 29).

The elongated parasites (Plate XXI, figs. 27, 28, 29) are also morphologically identical with the cultural forms; they reach a size of $9-11\mu$ in length by about 2μ in breadth; their anterior extremity is pointed; the posterior end is conical, as in the pear-shaped forms. Thus, as in the cultural forms, the various "organellae" may have different shapes and positions in different flagellate individuals.

Leishmania in the flagellate stage reproduces itself in fleas by longitudinal division (Plate XXI, fig. 28); this takes place in the same way as that described for the cultural forms. Either the flagellum, or the blepharoplast, or the nucleus may divide first; the plasma divides last. Two equal parasites are thus produced, and I have never met with unequal division.

In my numerous researches with naturally infected fleas I have very rarely found this flagellate stage; it has seemed to me that this stage may have some connection with the nutrition of the flea; I have more easily found it in fleas whose mid-gut still contained some undigested blood. This observation is of considerable value; if it should be confirmed it will provide a distinctive character between the developmental forms of *Leishmania* in fleas and the common *Herpetomonas* and *Crithidia* of insects, because these latter constantly show all stages, both flagellate and non-flagellate.

The flagellated *Leishmania*, after more or less active multiplication in the intestine of the flea, undergoes certain modifications in structure; the blepharoplast first approaches the nucleus, in some cases coming to lie beside it, the basal granule follows and, in this stage, the flagellum is attached along the anterior third of the body (Plate XXI, fig. 25). In successive stages the anterior, rounded extremity of the parasite becomes pointed and a very short flagellum projects from it; the posterior end, which was pointed, almost conical, becomes rounded and the parasite assumes a pear-shaped form (Plate XXI, fig. 30); later, the flagellum entirely disappears (Plate XXI, figs. 31, 32), the plasma appears of a red colour, the nucleus, the blepharoplast and the basal granule of a deep red colour; still later, the basal granule disappears, the blepharoplast becomes absorbed in the nucleus (Plate XXI, fig. 33); finally, the nuclear chromatin breaks up into numerous granules distributed in the plasma (post-flagellate stage) (Plate XXI, fig. 34). These post-flagellate bodies become provided with a cystic membrane and then the internal contents stain with difficulty. Cystic forms of flagellate Protozoa have been described; e.g., cysts of *Trypanosoma grayi* in the mid-gut of *Glossina palpalis*, which were discovered and described by Minchin. During encystment I have never ob-

served nuclear multiplication, such as has been described by Wenyon in similar forms of *Herpetomona's muscae-domesticae*.

The post-flagellate forms of *Leishmania*, which are expelled with the faeces, help to keep the species in existence and to spread it; this dispersal takes place through the infection *per os* of other fleas and perhaps also of other insects. My experiments, as also those of Archibald (1914), lead me to believe that these post-flagellate forms are also capable of infecting, by the alimentary tract, dogs and healthy children; this is an example of the contaminative mode of infection.

The description of the developmental cycle of *Leishmania* in my previous notes has been confirmed by Alvarez and Sergent.

Alvarez described the occurrence of *Leishmania*, in the pre-flagellate, flagellate and post-flagellate stages, in the intestine and faeces of fleas from a dog infected experimentally with infantile leishmaniasis; Sergent, in collaboration with Lhéritier and Lemaire, described *Leishmania* from the intestinal contents and faeces of fleas taken from a dog infected naturally with leishmaniasis. The studies of Sergent and his collaborators are highly important, because these writers, repeating my experiments with infected fleas, also succeeded in transmitting leishmaniasis to a healthy dog.

The study of the developmental cycle of *Leishmania* in the flea indicates the possibility that there may be two methods of infection of the vertebrate host; the method of inoculation, by the penetration of the parasite through the skin, and the method of contamination, by the post-flagellate forms *per os*.

This possibility of a double mode of infection explains not only the widespread distribution of leishmaniasis in dogs, but also the domestic localisation of the disease, members of the same family becoming infected by fleas, which themselves infect one another by means of the faeces.

LEISHMANIA, HERPETOMONAS, CRITHIDIA IN FLEAS.

The morphological study of the Protozoa of herpetomonad and crithidial types discovered in fleas reveals characteristics which differentiate them from the *Leishmania* which I have discovered in *Ctenocephalus canis* and *Pulex irritans*.

These distinctive characters are most evident in the flagellate stage; in this stage the dimensions of *Leishmania* in fleas, as given above, are very different from those of the various species of *Herpetomonas* and *Crithidia* which have been discovered up to the present in the *Pulicidae*.

The Flagellates observed by Sangiorgi in fleas (*Pulex serraticeps*) attained a length of 16.6μ with a breadth varying from $3-6\mu$. The flagellum in these forms is as long as, or longer than the body; *Herpetomonas ctenophthalmi* described by Mackinnon in *Ctenophthalmus agyrtes* also attains a length of 22μ , without including the flagellum, and a breadth of 2μ (Plate XXII, fig. 1); the *Herpetomonas* discovered by Wenyon in *Pulex irritans* is about 30μ long,

without the flagellum (Plate XXI, figs. 37, 38); *Crithidia pulicis* discovered by Porter in *Pulex irritans* may even reach a length of 65μ , including the flagellum (Plate XXII, fig. 12); and *Crithidia hystricopsyllae* discovered by Mackinnon in *Hystricopsylla talpae* is 18μ long, without the undulating membrane and the flagellum (Plate XXII, fig. 8). Forms notably larger than *Leishmania* are found also in *Herpetomonas ctenopsillae* described by Laveran and Franchini.

The anterior end of the young pear-shaped forms of *Leishmania* is rounded and is crossed by the flagellum, while in the Insectan Flagellates it is always pointed and drawn out along the flagellum. The posterior end of these forms in *Leishmania* is pointed and almost conical, while in the insectan flagellates it is rounded. *Leishmania* also sometimes shows the anterior end slightly pointed and the posterior end rounded (Plate XXI, fig. 31), but in this case the parasites are always short and thick, with a very short flagellum, and the blepharoplast is very close, or attached to the nucleus; these individuals represent only a stage leading on to the post-flagellate form.

The elongated forms of *Leishmania* in fleas, which are absolutely identical with the cultural forms, show a pointed anterior end, from which the flagellum projects, and a posterior end, which is abruptly truncated, almost conical. It is to be noted, however, that in the *Herpetomonas* described by Mackinnon in *Ctenophthalmus agyrtes* (Plate XXII, figs. 1-4), and by Wenyon in *Pulex irritans*, the posterior end is drawn out in a filiform prolongation (Plate XXI, figs. 37, 38).

Finally, *Leishmania* is differentiated from *Crithidia* by the complete absence of an undulating membrane (Plate XXII, figs. 8, 12, 13, 14, 15).

Herpetomonas and *Crithidia* of fleas, according to Mackinnon, Nöeller and Fantham, may show large forms, which are rounded or elongated (Plate XXII, figs. 5, 6, 11); in this case the ends are rounded or obtuse, the parasites possess a large nucleus and blepharoplast, and many granules distributed through the vacuolar plasma (Plate XXII, figs. 8, 11); the flagellum in these forms is very short and is continued into the body of the parasite as a conspicuous rhizoblast, more or less deeply coloured. I have never seen similar forms in the developmental cycle of *Leishmania* in fleas.

In studying the relations between *Leptomonas* of *Ctenocephalus canis* and *Leishmania* of human and canine origin, Chatton has recently observed that in cultures of *Leptomonas ctenocephali* large forms appear which he calls "aciculées flagellés," which present a body "rubanné" and "torder." We can thus distinguish at once between the cultural forms of *Leptomonas ctenocephali* and those of *Leishmania*.

Also the *Herpetomonas* discovered by Balfour in *Pulex cleopatrae* has certain stages, the dimensions and morphological characteristics of some stages of *Leishmania* in human and canine fleas; these, however, are differentiated morphologically in other flagellate stages, when the posterior end is filiform, as in the *Herpetomonas* found by Mackinnon in *Ctenophthalmus agyrtes* and by

Wenyon in *Pulex irritans*, while in *Leishmania*, as has been already stated, the posterior end is conical.

It can be deduced from these indications that an accurate study of the complete developmental cycle of the various *Herpetomonas* and *Crithidia* already described in fleas reveals certain morphological differences between these and the developmental forms of *Leishmania* in the same insects; on the other hand, the morphological study, if it be not supplemented by biological and experimental data, may be sometimes insufficient.

In this respect I repeat to-day what I have written ever since I began these studies in 1911, "given the constant presence of Protozoa, often morphologically similar, in the intestine of insects, anyone who studies their developmental cycle rather than their morphology is able, by means of experiments, to infer whether the said Protozoa are etiological agents of disease."

It is, in fact, necessary to bear in mind that the herpetomonad and crithidial forms found in the intestine of fleas are not always Insectan Flagellates, since developmental stages of trypanosomes which possess a herpetomonad or crithidial phase also occur in fleas. Minchin and Thomson found in the rat flea (*Ceratophyllus fasciatus*) the developmental stages of *Trypanosoma lewisi*; this parasite, in some of its forms, assumes all the characteristics of *Herpetomonas*; similar phases of *T. lewisi* have also been discovered in *Ctenocephalus serraticeps* (Nöeller), in *Pulex irritans* (Wenyon), and in *Xenopsylla cheopis*, experimentally infected; we cannot, therefore, exclude the possibility that they may be found in fleas infected in nature.

In reference to the relations between pathogenic forms of *Herpetomonas* and Trypanosomes the researches dealing with *Schizotrypanum cruzi* are interesting; in the digestive tract of *Triatoma megistus* taken from the houses of Minas Geraes, Brazil, Chagas observed Protozoa of the herpetomonad type; monkeys inoculated with the intestinal contents of these *Hemiptera* became infected with Trypanosomes; this discovery led Chagas to ascertain the existence of a trypanosomiasis previously unknown, which from its clinical symptomatology has been called "parasitic thyroiditis"; the parasite, on account of certain characteristics of its developmental cycle, has been called *Schizotrypanum cruzi*.

Results identical with those of Chagas have been obtained by Lafont in the island of Mauritius, and by Carini at San Paulo. Recently, also, Tejera when examining the intestinal contents of *Rhodnius prolixus* in Venezuela found these *Hemiptera* infected with flagellates which, when inoculated into animals, produced a trypanosome-infection identical with that caused by *Schizotrypanum cruzi*. These interesting investigations of Chagas into trypanosomiasis, or parasitic thyroiditis, indicate the importance which the researches of experimental protozoology upon the various *Herpetomonas* and *Crithidia* found naturally in fleas have in relation to leishmaniasis.

Since 1910 I have been seeking to solve the important problem of the pathogenic significance of the Protozoa of fleas taken from children and dogs

suffering from visceral leishmaniasis in the Mediterranean region. I have carried out experiments the results of which I will briefly relate. Fleas infected with parasites which I defined as of "leishmanial type," taken from dogs and children infected with leishmaniasis, were put upon healthy dogs. These parasites produced in healthy dogs an infection with the characteristic symptomatology of leishmaniasis; and characteristic leishmanial forms were found in the haematopoietic organs. Similar parasites in fleas were inoculated intraperitoneally into white mice and produced in these animals an infection which has been determined by microscopical examination of the internal organs to be identical with leishmaniasis of infantile or canine origin.

My investigations have been confirmed by Sergent, Lhéritier and Lémaire (1912).

While I, and subsequently Sergent and his collaborators, have made use of fleas taken from children and dogs infected with leishmaniasis, Sangiorgi (1911) began a series of experimental control investigations with a *Herpetomonas* in *Ctenocephalus serraticeps*, of unknown origin; unfortunately, the dogs of which he made use died a few days after the beginning of the experiment and before Sangiorgi was able to arrive at any conclusion.

A. Porter for a long period of time allowed fleas (*Pulex irritans*) infected with parasites of the *Herpetomonas*-type to suck blood from her arm every day, but she did not note any infection in herself. Nöeller, also, obtained negative results from the intraperitoneal inoculation of dogs with the intestinal contents of *Ctenocephalus serraticeps* infected with *Herpetomonas ctenocephali*; Negri obtained similar negative results from puppies (not published), and Chatton from white mice (*Mus albinus*).

To these negative results we can oppose a series of positive results.

Laveran and Franchini obtained in young rats an infection of the internal organs with parasites of a "leishmanial type," by inoculation, by natural means (puncture of the skin and by the digestive tract), making use of dog-fleas naturally infected with *Herpetomonas ctenocephali*. They obtained similar results also with rat-fleas (*Ceratophyllus fasciatus*) infected with *Herpetomonas pattoni*, with *Melophagus ovinus* infected with *Crithidia melophagia* and with *Anopheles* infected with *Crithidia fasciculata*, with *Sarcophaga hemorroidalis*, *Phlebotomus*, and *Blatta orientalis* infected with protozoa of herpetomonad and crithidial types, and finally by injecting cultures of *Herpetomonas ctenocephali* and of *Crithidia melophagia*.

The same writers prepared an emulsion in physiological saline solution of the internal organs of those rats experimentally infected with herpetomoniasis and injected it into dogs and monkeys, in which animals they obtained the reproduction of an infection not distinguishable from that which is observed after inoculation with *Leishmania*.

Fantham and Porter have experimentally demonstrated that Insectan Flagellates, such as *Herpetomonas jaculum* (Léger), of *Nepa cinerea*, *Herpetomonas ctenocephali* (Fantham), *Herpetomonas stratiomyae*, *Herpetomonas pedi-*

culi, and *Crithidia gerridis*, if inoculated intraperitoneally, or if ingested, can live and multiply in the blood and in the internal organs of young rats and of dogs and produce in these animals an infection indistinguishable from visceral leishmaniasis.

In later experiments Fantham and Porter have also demonstrated that a similar herpetomoniasis can be produced also in cold-blooded animals; they have, in fact, produced an infection with *Herpetomonas jaculum* and *Crithidia gerridis*, both by natural means (the digestive tract) and by experimental means (inoculation) in fishes (*Gasterosteus aculeatus*), frogs, toads, lizards (*Lacerta vivipara*) and in snakes (*Tropidonotus natrix*), parasites being present in the internal organs indistinguishable from those met with in leishmaniasis.

But in addition to these experiments the investigations of natural herpetomoniasis in animals are highly interesting.

Dutton and Todd in 1903 observed the natural occurrence of a *Herpetomonas* in the blood of a rat in Gambia; Fantham and Porter in 1909 again discovered this *Herpetomonas* in the blood of rats inoculated with spirochaetosis.

Sergent, Lhéritier and Lémaire in prosecuting their researches in Biskra on *Tarentola mauritanica* established that, in making cultures of the internal organs of *Tarentola* on blood agar, in 15.7 per cent. of the cases forms of *Herpetomonas* morphologically indistinguishable from the cultural forms of *Leishmania* were obtained; on this discovery they based the hypothesis that *Tarentola mauritanica* may be a "reservoir" host of *Leishmania tropica*.

Léger has recently affirmed that 2 per cent. of the saurians of the species *Anolis* show a blood infection of *Leptomonas* (*Leptomonas henrici*) which he believes to be of intestinal origin because the same *Leptomonas* was found in 50 per cent. of the individuals examined.

These facts now known to science show that in mammals, as in fishes, reptiles and amphibia (especially in those which are insectivorous) natural herpetomoniasis exist, or an infection with an insectan *Herpetomonas* may be induced, either by way of the digestive tract or by inoculation. Herpetomoniasis appears to be an infection which until to-day has not been distinguishable, either by the morphological and biological characteristics of the parasite or by its symptoms, from visceral leishmaniasis.

In my previous work on this subject I have already expressed my belief that the leishmaniasis are produced by Protozoa (*Herpetomonas* and perhaps also *Crithidia*) which have adapted themselves to live and multiply in vertebrate hosts; this adaptation is easier in the case of the vertebrate on which the insects are ectoparasitic.

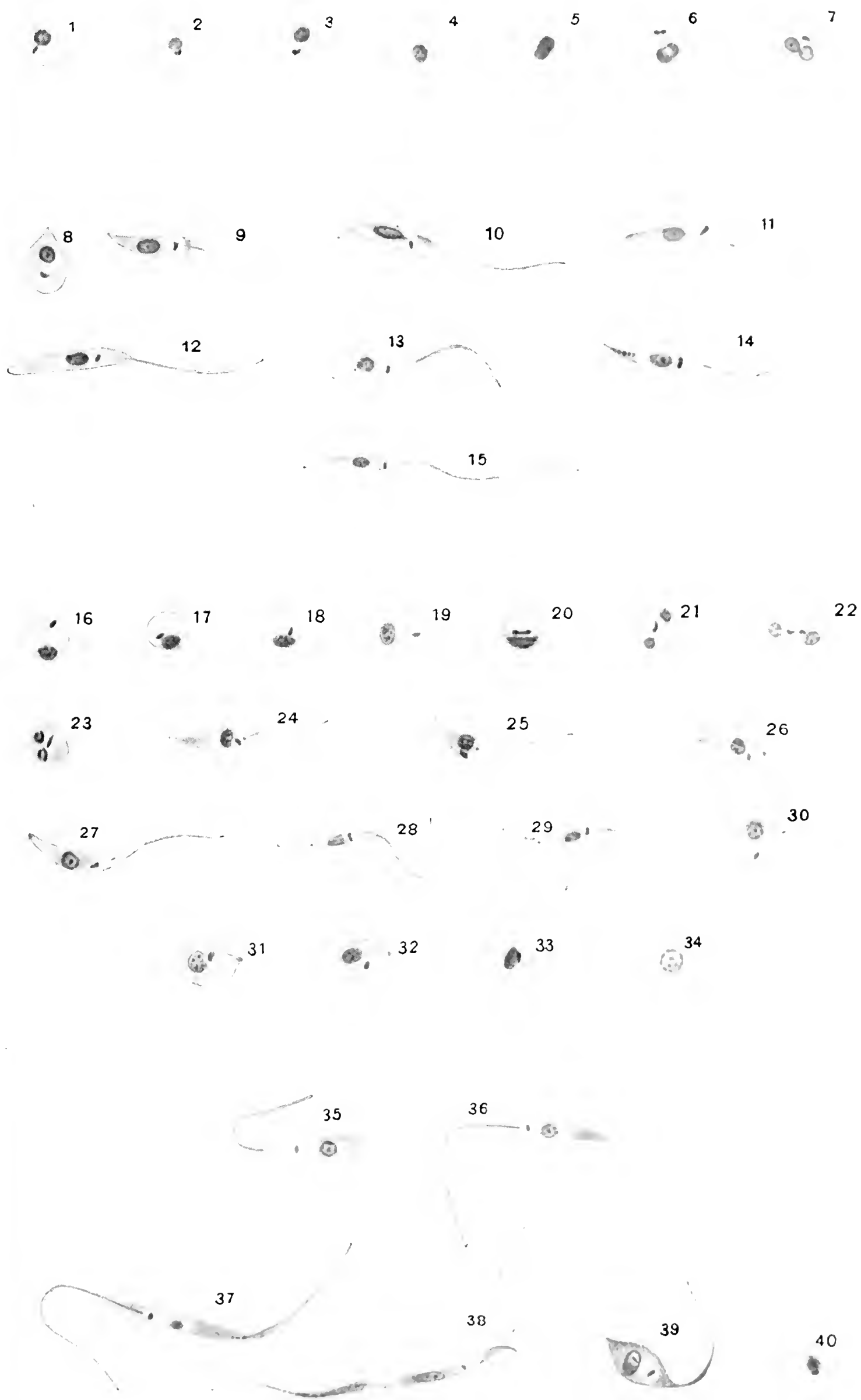
The same species of *Herpetomonas* of insects, under different conditions, may not have any pathogenic action, or may produce pathogenic effects in various degrees in different animal hosts. Referring especially to Mediterranean leishmaniasis this theory explains the acute forms, the chronic forms and the other spontaneously curable forms which have been distinguished in

this disease. I think that in the Mediterranean regions leishmaniasis may be much more frequent than has yet been ascertained, and that slight cases, not easily to be diagnosed, may be particularly frequent.

The facts obtained by my long-continued researches into visceral leishmaniasis in the Mediterranean regions, the epidemiological factors of the close relations between leishmaniasis in children and in dogs, the close relations of contact between children and dogs affected with leishmaniasis and fleas infected with parasitic protozoa which are morphologically and biologically indistinguishable from *Leishmania*, afford ever-increasing confirmation of the results of my investigations, and tend to prove that the visceral leishmaniasis of the Mediterranean is produced by a species of *Herpetomonas* or *Pulex irritans* and *Ctenocephalus canis* which has adapted itself to live in children and dogs, themselves the habitual hosts of these fleas.

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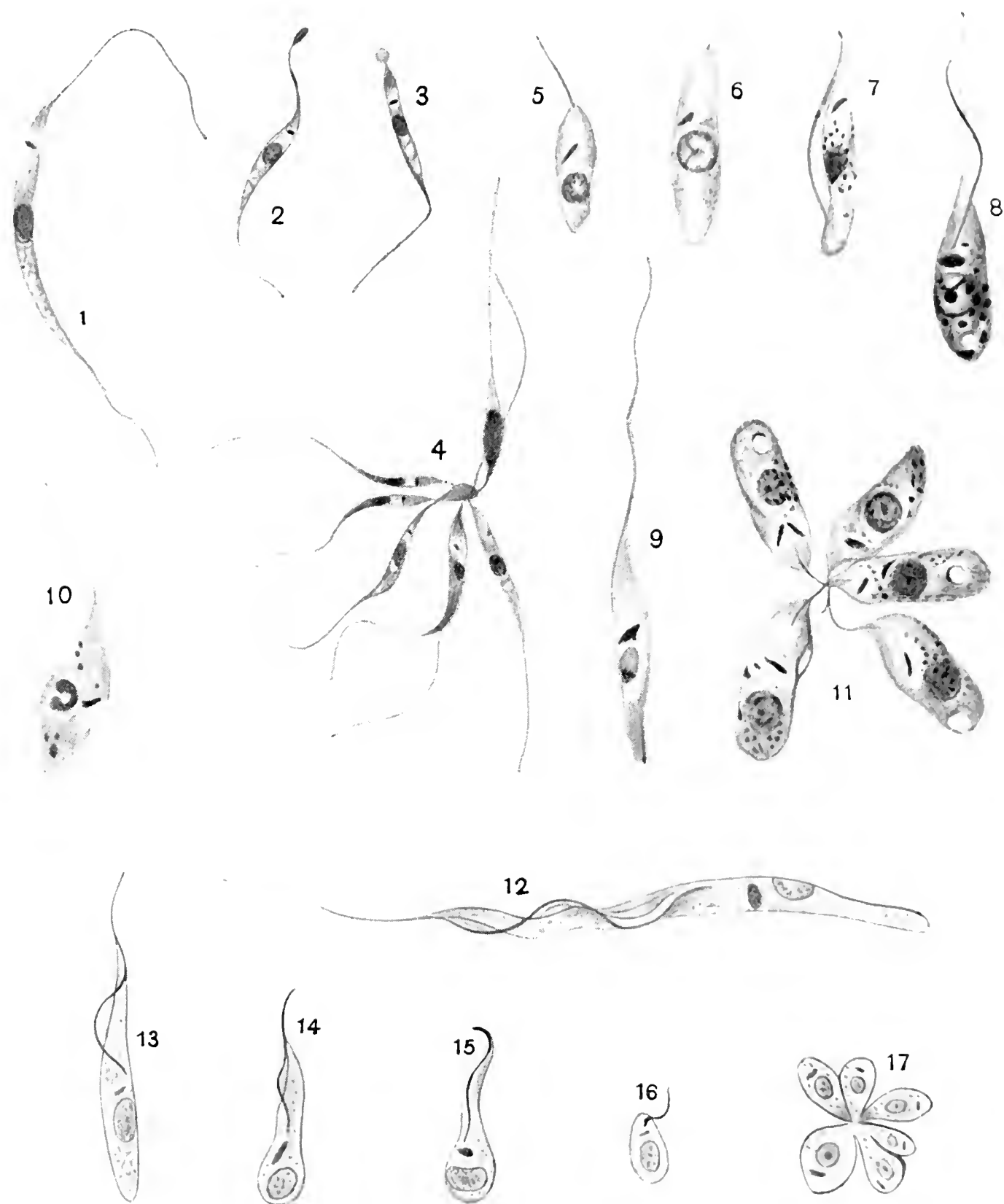
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EXPLANATION OF PLATES XXI AND XXII.

PLATE XXI.

- (a) *Leishmania* in the vertebrate host:
 Figs. 1-4. Non-flagellate stage.
 Figs. 5, 6. Showing homomorphous division.
 Fig. 7. Showing heteromorphous division.
- (b) *Leishmania* in cultures:
 Fig. 8. Young non-flagellate parasite.
 Fig. 9. Flagellate form derived from non-flagellate parasites
 Figs. 13, 14. Parasites commencing equal longitudinal division.
 Fig. 15. Form derived from flagellate parasites by longitudinal division.
- (c) *Leishmania* in *Ctenocephalus canis*:
 Figs. 16-19. Pre-flagellate stages.
 Figs. 20-22. Pre-flagellate stages showing equal longitudinal division.
 Figs. 24-29. Flagellate stage.
 Fig. 28. Parasite commencing equal longitudinal division.
 Fig. 30. Parasite approaching the post-flagellate stage.
 Figs. 31-34. Post-flagellate stage.
- (d) *Herpetomonas* in *Pulex irritans* (a specimen kindly sent me by Dr Wenyon):
 Figs. 35-39. Different stages of flagellate parasites in the flea's intestine.
 Fig. 40. Parasite in post-flagellate stage.

PLATE XXII.

- Figs. 1-7. *Herpetomonas ctenophthalmi* (after Mackinnon).
 Figs. 8-11. *Crithidia hystricopsillae* (after Mackinnon).
 Figs. 12-17. *Crithidia pulicis* (after Porter).

ENDOLIMAX KUENENI N.SP., PARASITIC IN THE
INTESTINAL TRACT OF THE MONKEY
MACACUS CYNOMOLGUS.

BY S. L. BRUG,

Major, Dutch-Indian Army Medical Service.

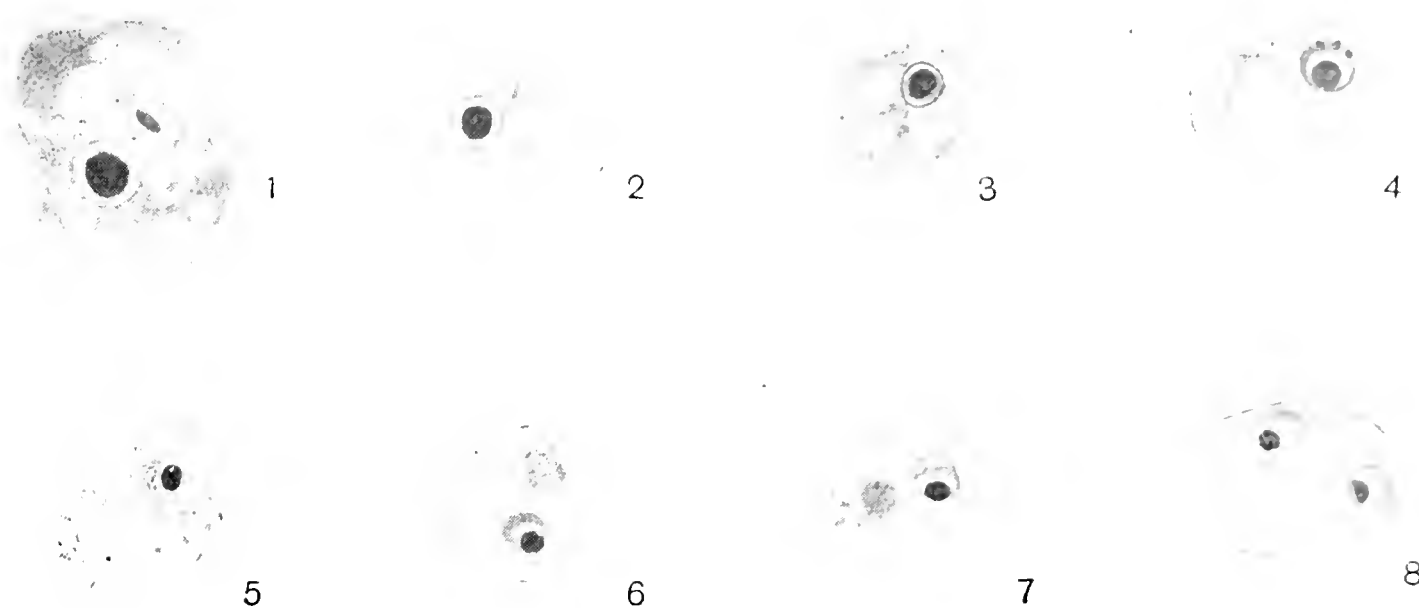
(*Centraal Militair Geneeskundig Laboratorium, Weltevreden, Java.*)

(With Plate XXIII.)

THIS amoeba was found in the large intestine of a *Macacus cynomolgus*, that was killed because it suffered from paralysis of the hind legs and emaciation. The autopsy showed these symptoms to be dependent on a general tuberculosis, in which, besides many other organs, the spinal matter was involved. Microscopic examination of the intestinal contents revealed the presence of motile amoebae and cysts. Except some small tubercular foci in the serosa no pathological lesions could be detected in the intestine. Close examination of the mucosa failed to reveal any ulceration; blood and mucus were totally absent.

When alive, the motile amoebae did not show a distinct nucleus. The ectoplasm was only visible where pseudopodia were being formed and it was apparently absent in the resting amoeba. The endoplasm was vacuolated, some vacuoles containing small food-particles, mostly bacteria. The amoebae measured 7–12 μ when rounded. The living cysts closely resembled iodine-cysts as described by Wenyon and O'Connor (1917). When the cysts were treated with Weigert's iodine solution, the resemblance with iodine-cysts was emphasised by the appearance of a dark reddish brown stained vacuole measuring $\frac{1}{3}$ – $\frac{2}{3}$ of the cyst's diameter. Moreover, just as in iodine-cysts, Weigert's solution failed to produce a clear nuclear picture. The diameter of the cysts varied between 7 and 10 μ ; they were round or oval-shaped.

On staining with Delafield's haematoxyline or according to Heidenhain's method as modified by Brug (1919), the amoebae showed the nuclear structure which Kuenen and Swellengrebel (1917) consider to be characteristic for the genus "*Endolimax*" (Plate XXIII, Figs. 1–3). The resemblance of the cysts still held good in stained films. The great majority of the cysts from the monkey were mononuclear, binucleate cysts were very rare (Fig. 8). The nucleus consisted of a darkly staining, large, homogeneous looking, excentrically situated, round caryosome, on one side surrounded by a crescent-shaped, less intensely coloured mass. The latter showed a granular structure more distinctly in



Endolimax kueneni (× 1500).

Fig. 1. Amoeboid form, Delafield's haematoxylin.

Figs. 2, 3. The same, iron haematoxylin.

Fig. 4. Cyst, Delafield's haematoxylin.

Figs. 5-7. Cysts, iron haematoxylin.

Fig. 8. Cyst with two nuclei, iron haematoxylin.

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Delafield preparations (Fig. 4) than in Heidenhain-stained films (Fig. 5). Caryosome and crescent were separated by a narrow clearly stained area. The iodophil vacuole appeared as an empty area. The cysts had a double contour, the outer limit of the cyst-wall being visible as a faintly coloured line.

The stained cyst almost always showed a structure which is absent in human iodine cysts. In the protoplasm there occurred a sharply limited area, round or somewhat irregularly shaped, whose periphery stained more darkly than the surrounding protoplasm whilst the centre stained like the protoplasm (Plate XXIII, Figs. 4, 6). In the minority of the cysts this structure stained homogeneously, without a clear centre (Fig. 7).

But for this darkly staining protoplasmic area in the cysts from the monkey's intestine these might be considered identical with the iodine-cysts of human origin. The constant presence of the darkly staining area in the monkey's parasite and its absence in the human parasite, suffices to differentiate these two amoebae. I therefore regard the former as a separate species, for which I propose the name *Endolimax kueneni*.

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ON SOME DIGENETIC TREMATODES IN JAPAN.

By HARUJIRO KOBAYASHI.

(Chosen Government-General Hospital, Korea, Japan.)

(With Plates XXIV—XXVI.)

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INTRODUCTION.

THE present paper is the outcome of my examination of a collection of trematodes from various parts of Japan (including Formosa). The species of monostome came from a turtle caught in Singapore. Of the material certain species are left unidentified chiefly owing to the paucity of specimens. It may be mentioned that in the following description the parasites are arranged according to their hosts.

At the outset I must express my thanks to Professors Ijima and Goto, Tokyo Imperial University, and Professors Miyajima and Yatsu, Keio University, for their generosity and suggestions.

I. MAMMALIAN TREMATODES.**(1) *Clonorchis sinensis* (Cobbold).**

It is well known that this species infests man, the cat, the dog and the pig. The rabbit, the guinea-pig and the rat can be artificially infested. Although it usually lives in the liver, yet sometimes it is found in the pancreas and even in the duodenum. As to the structure of this species the reader may refer to the papers by several authors, including my own, on its anatomy and life-history¹. It may here be mentioned that in my former paper the conclusion is reached that there is no difference at all between Looss's² *Cl. sinensis* and *Cl. endemicus*.

(2) *Microtrema truncatum* n. g., n. sp. (Pl. XXIV, fig. 1).

Microtrema n. g.

Diagnosis. Opisthorchiidae. Medium sized. Slightly flattened, but relatively thicker than other genera of the family. Taper anteriorly, truncated posteriorly. Cuticula densely spinose. Both suckers relatively small. Ventral and oral sucker widely separated. Intestinal caeca reaching to near posterior end. Excretory vesicle Y-shaped, stem branching into two arms near posterior end of receptaculum seminis; both arms are very long, run anteriorly close to the median line, and reach the exterior at a point near the intestinal bifurcation. Both testes lie symmetrically, slightly anterior to the branching of the excretory vesicle, and near intestinal caeca. No trace of cirrus-pouch. Ovary slightly anterior to level of both testes, lobate. Large receptaculum seminis present. Laurer's canal without opening. Vitellaria consist of several ascinous groups. Uterus largely anterior to ventral sucker, in the space between both intestinal caeca, and not extending posterior to ovary. Genital opening slightly anterior to ventral sucker. Eggs small and containing mature miracidia. Found in the liver of mammals.

¹ Kobayashi, H. (1917). On the life-history and morphology of the liver-distome (*Clonorchis sinensis*). *Mitt. Med. Fachsch. Keijo*, 1.

² Looss, A. (1907). Some parasites in the Museum of School of Tropical Medicine, Liverpool. *Ann. Trop. Med. and Parasit.* 1.

Type species: *M. truncatum* n. sp.

Microtrema truncatum n. sp.

Diagnosis. Size: 12–13 mm. length, 4–5 mm. breadth in the widest part, 1.5–2 mm. thick. Body tapers anteriorly, truncated posteriorly and commonly turned dorsally. Cuticula with densely lying needle-shaped spines. Oral sucker situate at anterior end. Ventral sucker slightly posterior to middle of body, and smaller than the oral. Testes lie symmetrically, or one being often slightly anterior, margin slightly sinuate. Vesicula seminalis short, spindle-shaped. Prostate glands numerous. Ovary with numerous fine lobes. Receptaculum seminis large. Laurer's canal without opening, ending blindly near dorsal body surface. Each vitellarium consists of about 10 ascinous groups. Uterus with numerous irregular loops, some of them barely reaching the intestinal caeca. Eggs brown, small, $0.026-0.03 \times 0.013-0.016$ mm. Found in the liver of hog.

Externally this species resembles the genus *Pachytrema* Looss, but from its internal structure it may be referred to *Metorchis truncatus* (Rud.) = *Pseudamphistomum truncatum* (Rud.).

DESCRIPTION. This species was found by Koidzumi (1911) in the liver of the pig in Formosa. It is 12–13 mm. long by 5–6 mm. wide in the widest part and 1.5–2 mm. thick in the thickest part.

Body somewhat tongue-shaped. The postero-lateral margins are parallel, the body narrows quickly anteriorly, and posteriorly it is truncated. Ventral surface slightly convex posteriorly. Oral sucker situated slightly ventrally and usually elongated transversely, it measures 0.6 mm. in width. Ventral sucker situated a little posterior to the middle of the body and 7–7.5 mm. from the anterior end; it is round or transversely elongated and measures 0.3–0.4 mm. Genital opening situated on median line and 1 mm. in front of ventral sucker.

The colour of the specimens in alcohol is pale, a few being brown. The uterus and the vitellaria can be seen shimmering through.

The cuticula is 0.02–0.05 mm. thick. It is armed all over with spines except postero-ventrally; the spines are specially numerous on the ventral surface; they are often arranged in threes or in fours; these groups are in turn disposed in transverse or diagonal rows. The spines are lance-shaped, 0.01–0.02 mm. in length by 0.004–0.006 mm. in breadth basally; those on the ventral surface are larger and project while those on the dorsal surface remain in the cuticula.

The three layers of the cutaneous muscles and dorso-ventral parenchyma muscles are all well developed.

The oral sucker is followed by a short prepharynx which leads to the pharynx; the latter measures 0.5×0.4 mm.; the oesophagus is short, being slightly shorter than the pharynx and posteriorly it widens and soon bifurcates into the intestinal caeca, which run parallel with the lateral margins of the

body down to the posterior end and terminate at the blind end, being turned slightly mesially.

The excretory pore is situated at the posterior end. The vesicle is Y-shaped, the stem occupying the posterior fifth of the body length and bifurcating near the posterior margin of the receptaculum seminis. The two arms run forward, parallel with each other, along the submedian line against the ventral side of the loops of the uterus, and near the anterior part of the intestinal caeca, they pass over the intestine, where the vesicula becomes continuous with the collecting vessel.

The testes are symmetrically disposed in the hindmost quarter of the body, each lying close to the corresponding intestinal caecum. They are of an irregular, slightly lobate form, with a diameter of about 0.5–0.7 mm. The vasa efferentia arise each from the inner side of the testis, and run antero-medially; at the anterior margin of the ventral sucker they unite into one tube and form the vesicula seminalis. The vesicula seminalis is spindle-shaped, being 0.6 mm. in length. It turns ventrally and is continuous with a short pars prostatica. The prostate glands lie on the ventral side of the vesicula seminalis and around the pars prostatica. The pars prostatica continues directly into the ductus ejaculatorius.

The ovary is situated on the median line, at the same level or slightly anterior to the testes. It is composed of numerous lobes and the whole organ presents a broad triangular shape, with a width of 0.8 mm. The oviduct arises from the dorsal surface of the ovary. The shell-glands are numerous, and lie in the antero-dorsal region of the ovary. The receptaculum seminis is an ellipsoid sac which lies posterior to the ovary; its diameter is 0.3–0.5 mm. The Laurer's canal, after making two or three turns, ends blindly in the dorsal parenchyma. The wall of the distal part of the canal has a peculiar vesicular structure. The vitellaria lie exterior to the intestinal caeca, and extend from the anterior extremities of the intestinal caeca to the level of the receptaculum seminis. Each of the vitellaria is composed of from nine to twelve clearly differentiated ascini. The yolk ducts run in a mesial direction from near the posterior end of the vitellaria and unite at the postero-dorsal margin of the ovary. The convoluted loops of the uterus occupy the entire region circumscribed by intestinal caeca laterally, and the testes and the ovary posteriorly. The vagina runs ventrally, parallel to the ductus ejaculatorius, and opens directly into the latter. The eggs (0.026–0.033 mm. \times 0.013–0.016 mm.) are provided with a clearly visible lid; those contained in the distal part of the uterus containing well developed miracidia.

- (3) **Eurytrema pancreaticum** (Janson) (Pl. XXIV, fig. 2); and
E. coelomaticum (Giard and Billet) (Pl. XXIV, fig. 3).

In 1907, Loos¹ described two species of distomes which infest the pancreas of cattle: viz. *Eurytrema pancreaticum* and *E. coelomaticum*. These had been regarded as synonymous. He distinguished them as follows:

1. *E. pancreaticum* is larger and thicker than *E. coelomaticum*; the body-length being more than 10 mm. long and the dorso-ventral thickness 2 mm., while *E. coelomaticum* is not more than 10 mm. in length and 1 mm. thick.

2. The tongue-like appendage at the posterior end of the body is more marked in *E. pancreaticum* than in *E. coelomaticum*.

3. In *E. pancreaticum* the oral sucker is remarkably larger than in *E. coelomaticum* and, in comparison with the body length, the distance between the suckers is greater.

4. *E. pancreaticum* has the distinctly lobate testes, but *E. coelomaticum* less distinct ones.

5. *E. pancreaticum* has larger vitellaria which contain more asci than *E. coelomaticum*. In the former they arise more anteriorly than in the latter.

6. *E. pancreaticum* lays eggs slightly larger than those of *E. coelomaticum*.

I have examined numerous specimens of distomes from the pancreas of *Bos taurus* and have observed these two distinct species as Looss distinguished them. My observations differ from those of Looss in the following respects:

1. The two forms are of about the same size, both measuring between 9.5–11.5 mm. in length. The thickness varies within the same range.

2. The tongue-like appendage on the posterior end seemed to me to have no specific value, individual variation being large.

3. The size and situations of the sucker are good criteria; for in *E. pancreaticum* the oral sucker is 1.8–2 mm. in diameter, while in *E. coelomaticum* it is remarkably small, being 1.3–1.5 mm. in diameter. The ratio of the diameters of both suckers is 10:7 in the former, and 8:7 in the latter.

The distance between the two suckers in the former is 2.2 mm. (measured from the posterior border of the oral sucker to the anterior border of the ventral sucker), while in the latter it is 1.7 mm. on an average.

4. Usually *E. pancreaticum* has distinctly lobate testes, while in *E. coelomaticum* the lobulation is less prominent, but as many intermediate forms exist in both species, this characteristic is of little use.

5. No distinction can be drawn from the relative situations of the vitellaria and the sizes of the eggs.

I distinguish the two species as follows:

E. pancreaticum (Janson). Body leaf-like, 10 mm. long by 4–6 mm. wide. Oral sucker 2.2 mm. in diameter; ventral sucker 1.4 mm. in diameter; the posterior situated at the posterior limit of the anterior half of the body. Testes and ovary usually obscurely lobate, the former consisting of 3–5 lobes,

¹ Looss, A. (1907), *l.c.*

the latter commonly of 3 lobes. Egg 0.045–0.055 mm. \times 0.03 mm. Parasitic in the pancreas of *Bos taurus*.

E. coelomaticum (Giard and Billet). Body leaf-like, 10 mm. in length by 4–6 mm. in width, anterior end ordinarily tapering more sharply than in *E. pancreaticum*. Oral sucker 1.3–1.5 mm. in diameter; ventral sucker 1.2–1.4 mm.; ventral sucker situated at the anterior third of the body. Testes and ovary usually indistinctly lobulated. Egg 0.045–0.05 \times 0.03 mm. parasitic in the pancreas of *Bos taurus*.

(Several forms were found in association with *E. pancreaticum*; they are distinguished from both the above-described species by their greater translucency and the slender uterus containing fewer eggs. Their real nature remains unknown.)

(4) **Eurytrema satoi** n. sp.¹ (Pl. XXIV, fig. 4).

Diagnosis. Body flat, elliptical, 6–6.5 mm. \times 2–3 mm., extremities tapering, posteriorly a tongue-like part is somewhat clearly marked. Oral and ventral suckers about equal in size; ventral sucker situated at the level of the anterior two-fifths. Intestinal caeca extend almost to the posterior end of the body. Testes adjacent to the postero-lateral margin of the ventral sucker. Testes entire, more or less symmetrically disposed. Ovary situated about the middle of the body. Vitellaria small, each consisting of groups of three to four ascini, extending posteriorly from the level of the ovary. Uterus with numerous loops. Egg small, 0.025–0.03 mm. \times 0.018 mm. with thick shell and flat lid. Habitat: the pancreas of *Macacus cynomolgus*.

DESCRIPTION. This species infests the pancreatic duct of *Macacus cynomolgus*. The specimens were collected by J. Sato in 1914. Several mature and immature specimens were obtained from a single host.

The body is dorso-ventrally flattened, giving a leaf-like appearance. The form is elongate ovoid or ellipsoid, the greatest width being reached at the posterior third of the body-length. The length is 6–6.5 mm. and the broadest part measures 2–3 mm. in a mature specimen. The lateral margins are gradually convergent anteriorly, while posteriorly they narrow abruptly and at the posterior end a tongue-like appendage is formed. In some cases the broadest part lies at the middle of the body and then the body narrows anteriorly and posteriorly in a like manner, though there is a tongue-like appendage, more or less well defined.

The cuticle is smooth. The oral sucker lies near the anterior end, its diameter is 0.5 mm.–0.55 mm. The ventral sucker lies in the anterior half of the body, and its size is equal, or slightly larger than that of the oral sucker, the diameter being 0.55–0.6 mm.

¹ Since this description was written, I have read the paper by A. Railliet, A. Henry et C. Joyeux (1912), Sur deux Trématodes des Primates, *Bull. Soc. Path. exot.* v. My species resembles *Eurytrema brumpti*, as described by these authors, but differs from the latter in the smaller number of its vitelline glands and in the weaker development of the uterus.

The pharynx lies immediately behind the oral sucker; its dimensions are 0.18 mm. \times 0.15 mm. The oesophagus is short, the length being 0.15 mm. The intestinal caeca reach almost to the posterior end of the body and terminate just in front of the tongue-like appendage. The course of the intestinal caeca is slightly sinuate and at the place where the vitellaria are present both caeca are bent inwardly.

The excretory pore opens at the posterior end. The median unpaired part of the vesicle runs anteriorly and at the level posterior to the ovary it divides into arms, which are bent at right angles. After passing over the intestinal caeca they again divide into anterior and posterior branches, which run along the lateral margins of the body and can be traced almost to the ends of the body.

The testes lie immediately postero-lateral to the ventral sucker, their external borders touching or overlapping the intestine. They are round or slightly lobed into 3–5 lobes, their diameter is about 0.4–0.5 mm.; they lie at about the same level.

The cirrus-pouch lies anterior to the ventral sucker and slightly to the right of the median plane. Its size is 0.7 mm. \times 0.2 mm. The vesicula seminalis and the pars prostatica make one or two turns. The genital pore opens on the median line, slightly posterior to the bifurcation of the oesophagus.

The ovary lies at about the middle part of the body, and slightly left of the median line. It is slightly elongated transversely, the transverse diameter being 0.18 mm. The vitellaria lie exterior to the intestinal caeca, at the level of the ovary and extending posteriorly for a short distance, and often extend over the intestine. They are composed of three or four ascini. The uterus lies in the space between the two intestinal caeca, often reaching outside the latter. It first runs posteriorly and then anteriorly, passing through the space between the left testis and the ventral sucker and runs anteriorly along the left side of the ventral sucker. At the anterior border of the ventral sucker it turns mesially and after forming a short vagina, opens exteriorly near the male genital pore. Along the whole course the uterus forms numerous lateral loops. The eggs are fairly numerous, their size is 0.025–0.03 mm. \times 0.018 mm. The shell is relatively thick and the lid is flat.

Younger specimens measure 1.5 mm. \times 0.45 mm.; they have an ellipsoidal shape, the body tapering at both ends. Both the suckers measure 0.2 mm. in diameter. The ventral sucker lies at the middle of the body. The alimentary canal, the excretory vesicle and the immature genital organs can be recognized.

(5) **Dicrocoelium macaci** n. sp. (Pl. XXIV, figs. 5–8).

Diagnosis. Body-shape variable, the broadest part being near either anterior or posterior part; size 4.5–5 mm. \times 1–1.5 mm. Cuticle with minute protuberances, densely crowded on the anterior body surface. Ventral sucker slightly larger than the oral. Intestinal caeca terminating at the posterior third of the body, usually unequal in length. Testes lie slightly posterior to

the ventral sucker, lying symmetrically or obliquely, margin irregularly sinuate. Ovary situated slightly posterior to the left testis, rounded or weakly sinuate. Vitellaria extending throughout the middle third of the body-length. Eggs 0.042–0.045 mm. \times 0.025 mm. Habitat: the liver of *Macacus speciosus*.

DESCRIPTION. This species was found in the liver of a Japanese monkey, *Macacus speciosus*. Several specimens were collected by M. Miyajima in 1910.

Body lanceolate, 4.5–5 mm. in length by 1–1.5 mm. in width in the broadest part¹.

The greatest width is reached at the anterior third or fourth of the body-length, where the ovary and the testes are situated (Pl. XXIV, figs. 6 and 7). It narrows more abruptly anteriorly than posteriorly. In some specimens, the broadest part is reached at the posterior third of the body-length (like *Dicrocoelium lanceolatum*) (Pl. XXIV, figs. 5 and 8).

The cuticle is provided with numerous fine conical protuberances especially in the anterior part of the body. These become gradually indistinct posteriorly. The oral sucker lies at the anterior end. It has a round form, and a diameter of 0.25 mm. The ventral sucker is situated at the anterior sixth or seventh of the body-length and is slightly larger than the oral, the diameter being 0.32 mm.

The oral sucker is continued directly to the pharynx which is 0.06 mm. in length. The oesophagus is 0.1–0.2 mm. in length and bifurcates into the intestinal caeca midway between the oral and ventral suckers. The intestinal caeca are slender and end at the posterior third of the body-length. They often differ from each other in length.

The excretory pore opens at the posterior end. The unpaired part of the vesicle runs anteriorly and reaches almost as far as the posterior border of the ovary, where it divides into two arms. Both arms turn laterally, almost perpendicularly, and pass exterior to the intestinal caeca. Each of them again divides into anterior and posterior branches, which run along the lateral body margin.

Several gland cells lie on the lateral side of the oesophagus. The glandular ducts run independently forwards and find their opening on the dorsal side of the oral sucker. The contents of these gland-cells and their ducts are eosinophile.

The testes lie directly posterior to the ventral sucker. In certain elongated specimens in which the broadest part is found anteriorly, the right testis lies anterior to the left (Pl. XXIV, fig. 7). In certain other specimens, which have a similar shape to the above, the testes lie side by side (Pl. XXIV, fig. 6). In those specimens, in which the broadest part of the body is situated at the posterior region, both testes lie usually side by side in the same level (Pl. XXIV, figs. 5 and 8). The shape of each testis is lobate or irregularly square, with a diameter of about 0.4 mm.

¹ Measurements from specimens preserved in alcohol, after fixation in sublimate solution under slight pressure.

The cirrus-pouch lies in the region antero-dorsal to the ventral sucker and opens slightly posterior to the place of bifurcation of the intestine. The pouch contains a tubular, winding vesicula seminalis, a short pars prostatica and a long ductus ejaculatorius. The latter may be evaginated exteriorly as the penis.

The ovary lies posterior to the left testis, near the median line. It has an ellipsoid or slightly lobate form, 0.2 mm. in diameter. The oviduct rises at the postero-lateral margin of the ovary. The receptaculum seminis is a small round sac, 0.06 mm. in diameter. Laurer's canal is slender and ends blindly near the dorsal surface. The vitellaria lie exteriorly to the intestine, extending longitudinally in the middle third of the body length. The uterus lies between the intestinal caeca and runs posteriorly. As it approaches the posterior end of the body it turns forwards and passing between the testes, and the ventral sucker, the uterus is continued to the vagina, which opens to the exterior, alongside the male genital pore. Along the course of the uterus numerous smaller loops are met with. The uterus occupies almost the entire space between the ovary and the intestinal caeca.

The eggs are numerous; they measure 0.042–0.045 mm. long by 0.025 mm. wide and each has a relatively thick elliptical shell.

Here it may be desirable to state the probable affinities of this and other species. There are seven genera of mammalian Dicrocoeliinae: *Athesmia*, *Lyperosomum*, *Brodenia*, *Eurytrema*, *Platynosomum*, *Paradistomum*, and *Dicrocoelium*. Of these, three genera (*Athesmia*, *Lyperosomum* and *Brodenia*) may readily be distinguished, one from another, by the following characteristics:

1. In *Athesmia*, the vitellaria are absent on one side.
2. In *Lyperosomum* the body is elongate, and the testes are situated mesially one behind the other.

3. *Brodenia* has a remarkable serration in the middle part of the body.

In the remaining four genera, the differential characters are as follows:

1. In *Eurytrema*, the body is broad and the testes lie side by side at the same level.

2. In *Platynosomum*, the body is lancet-shaped and the widest portion is situated in the middle of the body. Both the testes and the ovary are also found in the widest part. The former are disposed symmetrically.

3. *Dicrocoelium* is lancet-shaped, and the widest portion is situated rather posteriorly. One of the testes lies a little anterior to the other.

4. *Paradistomum* is oval in outline, broadest near the posterior end, and the testes lie side by side.

All the above four genera have a smooth cuticle, excepting *Dicrocoelium concinnum* Braun, whose cuticle is armed with spines. Looss considers this species as belonging to a new genus, because of the spined cuticle.

In some specimens of *Dicrocoelium macaci* the widest portion is at the

level of the testes and ovary, as in *Platynosomum*; in others, however, the widest portion is in the posterior region as in *Dicrocoelium* and *Paradistomum*. The position of the testes varies considerably; in some specimens they lie symmetrically, as in *Platynosomum*, *Eurytrema*, and *Paradistomum*, while in others they lie obliquely. The cuticle of *D. macaci* is not smooth, being covered with fine conical protuberances. Though these protuberances differ from the ordinary spines of Digenea, yet they may be included in the same category. From these characteristics it seems reasonable to establish a new genus for this species. Since the shape of the body and the situation of the testes are not constant in this species, I am rather inclined to believe that the diagnoses of the above-mentioned genera are in need of revision, even to the extent of abolishing certain genera of this group. This species is included only provisionally in the genus *Dicrocoelium*.

(6) **Fasciola hepatica** Linnaeus.

This species is often found in the liver of *Bos taurus*. As a rule few flukes are found in each host.

(7) **Fasciolopsis buski** (Lankester) (Pl. XXV, figs. 2 and 3).

This species is often found in the swine, as well as in the human intestine, in Eastern Asia. In Japan, so far, it has not been found in man. M. Koidzumi has collected two specimens of this species from a Formosan pig (1911). Later K. Nakagawa (1916) kindly sent me several specimens from pigs in Formosa. As a result of my examination, several interesting features, hitherto unrecorded, have been revealed.

The surface of the body is covered with large, closely-set, scale-like spines (Pl. XXV, fig. 3). It may be mentioned that Leiper¹ states that the human species, *Fasciolopsis buski*, alone, is destitute of spines, while all the other species of this genus are spinose. He also remarks that the spines of this genus are easily shed. Goddard² recently observed that spines are present in this species.

The body varies remarkably in size, according to the degree of contraction.

The oral sucker has a longitudinally elongated, conical form. The posterior part of the sucker is surrounded by remarkably well-developed annular muscles, Odhner's prepharyngeal sphincter³. The prepharyngeal muscles are directly attached to the sucker. There is a short prepharynx, the posterior half of which is surrounded by well-developed annular muscles. These annular muscles are attached posteriorly directly to the pharynx, as the pharyngeal sphincter is, in its turn, attached to the oral sucker. The pharynx has a

¹ Leiper, R. T. (1913). Observations on certain helminths of man. *Trans. Soc. Trop. Med. and Hygiene*, vi.

² Goddard, F. W. (1919). *Fasciolopsis buski*, a parasite of man as seen in Shaohing, China. *Journ. Parasitol.* v. No. 4.

³ Odhner, T. (1902). *Fasciolopsis buski*. *Centralbl. f. Bakt. etc.* xxxi.

spherical shape. The oesophagus is longer than the prepharynx. The uneven-walled intestinal caeca take a winding course but do not branch.

The excretory vesicle (Pl. XXV, figs. 2 and 3) has most remarkable features. The pore opens on the dorsal surface near the posterior end, and is connected to the vesicle by a short canal. The vesicle has a structure similar to that of the Echinostomidae and the Psillostomidae. The median trunk of the vesicle runs straight forwards to the posterior border of the shell-gland, where it divides into two, each branch running along the lateral margin of the shell-gland. At the anterior margin of the gland they unite again into a single tube, which runs anteriorly. At the posterior end of the ventral sucker it is continuous with the lacunae. The lacunae surround all the organs in the interior part—the two suckers, the prepharynx, the pharynx, the oesophagus and the terminal parts of both genital ducts. Four lateral branches arise on each side from the median trunk of the excretory vesicle, two in the region posterior to the shell-gland, one from each ramus alongside the shell-gland, and one anterior to the shell-glands. There is a closely set network of tubules under the cuticle of the entire body surface, which network is continuous with the vesicle, morphologically the former being a part of the vesicle. The network is more abundant ventrally and anteriorly.

The vesicula seminalis is very long and takes a peculiar spiral course with three turns. At the anterior end of the third turn, it has a caecal appendage which runs posteriorly along the wall of the vesicula seminalis proper, and terminates near the posterior end of the latter. The walls of these two parts are formed of epithelial cells. Externally they are surrounded by the long cirrus-pouch. Almost no space exists between the epithelium and the muscle layers of the cirrus-pouch.

Anterior to the vesicula seminalis with its caecal sac, the cirrus-pouch runs directly forwards, passing along the dorsal surface of the ventral sucker. The epithelial cells at this part are elongated, and this portion was taken for the pars prostatica by Odhner¹, but his conclusion may be doubted, since, as Odhner himself observes, it lacks the glandular cells around it. The real pars prostatica lies further forward, in the antero-dorsal region of the ventral sucker, and is delimited from the vesicula seminalis by a constriction. Numerous cells of the prostate glands lie around it. The pars prostatica is continued distally into a relatively long ductus ejaculatorius, which opens to the exterior. The wall of the ductus ejaculatorius is armed with stout spines. The wall of the cirrus-pouch is closely attached to the male duct, contrary to Odhner's figure.

This, with other species of *Fasciolopsis*, has no affinity with the genus *Fasciola*, as has been supposed by previous authors. The following differences are presented by the two genera:

1. *Fasciola* infests the liver, while *Fasciolopsis* is found exclusively in the intestine.

¹ Odhner, *l.c.*

2. Radical differences are manifested in the structure of the cirrus-pouch and the vesicula seminalis.

3. The ventral sucker of *Fasciolopsis* has a peculiar bulging on the posterior wall, while *Fasciola* has none.

4. The oral sucker of this species has a peculiar prepharyngeal sphincter which is wanting in *Fasciola*.

5. In the parenchyma of *Fasciolopsis* numerous well-developed, longitudinal parenchyma muscles are present, while in *Fasciola* they are wanting.

6. *Fasciola* has branched intestines, contrary to *Fasciolopsis*.

7. Remarkable differences are presented in the excretory vesicle of *Fasciolopsis*.

These and other characteristics show that *Fasciolopsis* has affinity with the Echinostomidae, and even may be considered as an aberrant form of the Echinostomidae.

Consequently, the generic and specific diagnosis of this parasite should be thus modified.

Fasciolopsis Lss., emend.

Diagnosis. Echinostomidae. Body large, flat. Cuticle with scale-like spines. Without well-developed, spinose head collar. Oral sucker with peculiar sphincter muscles along its posterior margin. Excretory vesicle with peripheral network. With long cirrus-pouch, which reaches far behind the ventral sucker. Testes lying one behind the other and terminally branched, likewise the ovary. Vesicula seminalis with spiral wall and a peculiar blind sac. Vitellaria well developed. Uterus relatively long, with several loops. Parasitic in the intestine of mammals.

Type species: *F. buski* (Lank.).

Fasciolopsis buski (Lank.), emend.

Diagnosis. Size 24–27 mm. \times 5.5–12 mm. Cuticle with spines, more numerous on the ventral body surface. Oral sucker small. Ventral sucker much larger than the oral, with peculiar posterior cup-like evagination. Longitudinal parenchyma muscles remarkably well developed. Intestinal caeca take a somewhat winding course. Excretory vesicle with a median stem and numerous lateral branches, subcutaneous network, and a large lacuna between the two suckers. Cirrus-pouch is canal-like and extends about one-fourth of the body-length. The greater part of the vesicula seminalis lies posterior to the ventral sucker. Cirrus with fine spines projecting in the lumen. Testes lie in the posterior half of the body. Ovary lies directly in front of the anterior testes, slightly right to the median line. Vitellaria extend from the ventral sucker to the posterior end of the body. Eggs large, elliptical with thin shell, 0.12–0.13 mm. \times 0.07–0.08 mm. Parasitic in the intestine of man and pig.

Recently Rodenwaldt¹ has described a new species, *Fasciolopsis fuelleborni*. I have reason to believe that this is *F. buski*. *F. fuelleborni* is distinguished from Odhner's *F. buski* principally by the absence of the caecal sac of the vesicula seminalis. He may have mistaken the caecal sac for the cirrus-pouch. Should his observations be correct, *F. fuelleborni* would rather belong to some genus other than *Fasciolopsis*. *F. rathouisi* Poirier², and *F. goddardi* Ward³ may not be good species.

(8) **Paragonimus westermani** (Kerbert).

This species is parasitic in the lung or the viscera of man, the dog, the cat, the tiger and the pig. I have dealt with the structure and the life-history of this species in other communications⁴.

(9) **Fischoederius elongatus** (Poirier), and **Paramphistomum** sp.

These two species are found very frequently and numerous in the first and second stomachs of *Bos taurus*. It is certain that there are several species of Paramphistomidae in Japanese cattle; they need further investigation.

(10) **Watsonius macaci** n. sp.⁵

Diagnosis. Size 11.3 mm. \times 6.5 mm. Oral sucker small, 0.75 mm. in length. Posterior sucker (acetabulum) very large, 2 mm. in diameter. In the caecum of *Macacus cynomolgus*.

DESCRIPTION. The following description is based on some ten specimens collected from the caecum of *Macacus cynomolgus*.

Body flattened; broadest part at the level of the posterior fifth of the body; posterior end rounded; anteriorly the body narrows, the narrowing becoming abrupt at the anterior sixth of the body-length. Dorsal and ventral surfaces are slightly convex. Specimens fixed by heat are 11.3 mm. in length, 6.5 mm. in breadth, and 2 mm. in thickness; specimens fixed with sublimate are 2-3 mm. shorter than those killed by heat. Posterior sucker lies at posterior extremity of body; round in contour, margin elevated; diameter 2 mm. Oral sucker lies at the anterior body extremity; its anterior half cylindrical, its cross section being ring-like; posteriorly it flattens and shows a crescent shape in transverse section; its size is 0.015 mm. \times 0.45 mm. Postero-laterally a pair of spherical suckorial pouches are present, 0.4-0.5 mm. in diameter.

¹ Rodenwaldt, E. (1909). *Fasciolopsis fuelleborni* n. sp. *Centralbl. f. Bakteriolog.*, etc. L.

² Poirier, S. (1887). Note sur une nouvelle espèce de Distome, parasite de l'homme, le *Distomum Rathouisi*. *Arch. zool. expér. et gen.* (2), v.

³ Ward, H. B. (1909). *Fasciolopsis buskii*, *F. rathouisi* and related species in China. *Stud. Zool. lab. Univ. Nebraska*, No. 94.

⁴ Kobayashi, H. (1918). Studies on the lung-fluke in Korea. 1: On the life-history and morphology of the lung-fluke. *Mitt. Med. Hochsch.* II. (1919), 2: Structure of the adult worm. *Ibid.* IV.

⁵ It is very probable that this species is identical with *Watsonius watsoni* collected by Joyeux from *Cercopithecus callitrichus* (A. Railliet, etc. *Bull. Soc. Path. exot.* v.).

Oral sucker continues into the oesophagus at the posterior median point of the former. Oesophagus 1.6 mm. in length; its lumen is narrow and posteriorly it becomes wider and its posterior fourth forms the pharyngeal bulb. The pharyngeal bulb has a diameter of 0.45 mm. and it directly divides into the caeca. Intestinal caeca broad, running directly and somewhat close to the submedian line and terminating posteriorly immediately behind the posterior testes, *i.e.* at the level of posterior two-fifths of the body-length.

The excretory vesicle lies between the anterior margin of the posterior sucker and the shell-gland; posteriorly it becomes a narrow canal which opens at the point 1 mm. from the posterior extremity. The whole organ lies posterior to Laurer's canal. Two vessels arise from the vesicle and run anteriorly along the inner side of the intestinal caeca.

The genital pore opens in a slight prominence on the median line, slightly posterior to the oral sucker.

One testis lies behind the other at about the middle of the body in the space between the intestinal caeca, partly overlapping the latter. The testes are somewhat separated one from the other. Each testis has four or five lobes; when four-lobed it is cross-shaped, and inclined at an angle of 45° to the longitudinal axis. Each testis is about 1.5–1.8 mm. in diameter. The vesicula seminalis is a broad canal, lying along the median line between the anterior testis and the pharyngeal bulb; it has many lateral loops. At the anterior part of the pharyngeal bulb it continues into a relatively thick *pars muscularis* which runs directly toward the anterior end and soon narrows, forming the *pars prostatica*. The *pars prostatica* continues directly into the *ductus ejaculatorius*, which unites with the vagina and thus a very short *ductus hermaphroditicus* is formed. Around the *ductus ejaculatorius*, the vagina and the *ductus hermaphroditicus* certain muscles are present which represent a primitive cirrus-pouch.

The ovary lies slightly in front of, and to the right of the posterior sucker. Its contour is round or ellipsoidal; its diameter is 0.5 mm. The shell-glands are attached between the point of bifurcation of the intestinal caeca and the anterior margin of the posterior sucker. They lie exterior to the intestinal caeca, partly overlapping them. Each consists of from 8 to 12 groups. The uterus forms several loops between the intestinal caeca and opens into the vagina at the level of the *ductus ejaculatorius*. The egg is ellipsoid; its size is 0.12 mm. \times 0.06 mm. The shell is not thick, and is operculated.

II. AVIAN TREMATODES.

(11) *Prosthogonimus japonicus* Braun.

One specimen was collected by O. Takagi, in a hen's egg, at Ibaraki Prefecture, 1915. Body 4 mm. in length by 2 mm. in width.

III. REPTILIAN AND AMPHIBIAN TREMATODES.

(12) *Polyangium miyajimai* n. sp. (Pl. XXV, fig. 4).

Diagnosis. Size 10–11 mm. \times 2 mm. Oral sucker round, 0.3 mm. in diameter. Intestinal caeca with sinuate wall. No trace of pharyngeal bulb. Eggs 0.07 mm. \times 0.04 mm. Habitat: in the intestine of *Chelone midas*.

DESCRIPTION. This species was found infesting the intestine of *Chelone midas* at Singapore in 1914. The parasite was collected by M. Miyajima, M. Koidzumi and R. Takano. The specimens were fixed by heat.

The size of a mature specimen is 10–11 mm. \times 2 mm. The lateral margins of the body are almost parallel, but posteriorly the body slightly narrows. The posterior end is pointed while the anterior end is rounded. The cuticle is beset with fine, closely set processes, but they are often insignificant. The oral sucker has a round outline, the diameter being 0.3 mm. The oesophagus is narrow, the length being 2 mm. There is no trace of the muscular thickening in the posterior part of the oesophagus. The intestinal caeca are broad and simple and run straight along the lateral margin of the body and terminate near the posterior end.

The excretory pore lies medianally on the dorsal side near the posterior end of the body. The unpaired part of the vesicle runs directly almost to the posterior part of the ovary.

The testes lie 0.2 mm. apart, one behind the other, in the space between the intestinal caeca, the hindmost being situated 2 mm. from the posterior end of the body. They have a round shape, their diameter is 0.8 mm. and they are directly attached laterally to the intestine, the latter often bending exteriorly at the point of attachment. The vesicula seminalis is long and runs in the median plane, slightly bending laterally. Anteriorly the vesicula seminalis reaches the level of the bifurcation of the oesophagus, where it joins the pars prostatica. The pars prostatica is a straight canal; lying in the median line, near the ventral surface.

The ovary lies directly behind the posterior testis, slightly to the right of the median line. The outline of the ovary is somewhat round; the diameter being 0.3 mm. Posterior to the ovary lies the compact mass of the shell-gland. The vitellaria occupy the posterior three-fifths of the body, lying mostly outside the intestinal caeca; a small part, however, is found inside the intestinal caeca posterior to the ovary. The uterus, with relatively few loops, lies between the intestinal caeca. Anteriorly the uterus runs straight along beneath the pars prostatica and the oesophagus, and opens to the exterior through a short vagina.

The genital opening lies in the ventral median line, 0.5 mm. from the anterior end of the body.

The egg has a size of 0.07 mm. \times 0.04 mm., with relatively thick wall and a distinct lid. The mature shell-wall is brown in colour.

(13) **Cricocephalus koidzumii** n. sp. (Pl. XXIV, fig. 9).

Diagnosis. Resembles *Cr. albus*, but differs from it by (1) its smaller size, 3.5–3.8 mm. \times 0.8–1 mm., (2) its very sinuate testes, (3) its having no trace of a pharyngeal bulb. Habitat: in the intestine of *Chelone midas*.

DESCRIPTION. This species was found in the stomach of *Chelone midas*, captured at Singapore. The specimens were collected by M. Miyajima and others and were fixed by heat.

The size is 3.5–3.8 mm. \times 0.8–1.0 mm. Body somewhat flattened dorso-ventrally; lateral margins ventrally curved except in the cone-shaped part. The posterior end truncated; the broadest part is near the posterior end and the body either slightly narrows anteriorly, or both lateral margins run almost parallel; at the "shoulder" it abruptly narrows and forms a cone-shaped anterior end; protuberant postero-laterally.

The oral sucker lies near the anterior end of the body, its diameter being 0.3 mm. The cuticle of the body surface is smooth. The oesophagus is 0.5 mm. in length. The intestinal caeca reach almost to the posterior end of the body. Each has numerous (20–30) diverticula, which undergo further subdivision.

Excretory pore opening at the posterior end of the body. The unpaired part of the vesicle runs forward, beyond the level of the posterior margin of the testes, and then divides into two.

Both testes lie symmetrically in the posterior part, their distance from the posterior end being 0.4 mm. They lie exterior to the posterior trunk of the intestine. They have a somewhat lobate or sinuate form, 4–5 lobes being present. The width of each testis is about 0.2 mm. The intestinal caeca bend inwards at the place of attachment of the testes. The vesicula seminalis lies in the anterior part of the posterior half of the body; it is a broad canal with numerous irregular lateral bends. The cirrus-pouch is spindle-shaped and continues anteriorly into the ductus ejaculatorius.

The ovary lies slightly anterior to the level of the testes, somewhat to the right of the median line. It has somewhat irregular lobes, and its diameter is 0.15 mm. Postero-medially to the ovary are the shell-glands. The vitellaria are small, lying exteriorly to the intestinal caeca. They extend shortly anterior to the level of the ovary. Each gland consists of about 15 groups. The uterus is surrounded by the intestinal caeca, the ovary, and the posterior end of the ductus ejaculatorius. It has many transverse loops which lie in close contact with one another. The vagina is very well developed, running along to the left of the ductus ejaculatorius. The distal part of the vagina has long spines in its cuticular wall.

The genital opening lies left of the median line 0.7 mm. from the anterior end of the body. A short genital sinus is present.

The egg bears long, whip-like appendages at both poles. The size of the normal egg is 0.02 mm. \times 0.01 mm., while the length of each appendage is

about 0.12 mm. The appendages develop with the growth of the egg-shell, young eggs showing small protuberances only.

(14) **Octangium takanoi** n. sp. (Pl. XXV, fig. 5).

Diagnosis. Size 5.5 mm. \times 1.2 mm. in largest specimens. Oral sucker somewhat ellipsoidal. Vitellaria extend posteriorly from level of anterior testis. Eggs 0.07–0.075 mm. \times 0.045 mm. Habitat: in the intestine of *Chelone midas*.

DESCRIPTION. This species was found in the intestine of *Chelone midas*, captured at Singapore in 1914. The specimens were collected by M. Miyajima and others and fixed by heat.

Body elongate, breadth uniform in main part of the body, slightly narrowing anteriorly and posteriorly. The general appearance resembles that of *Octangium hasta* Looss. The size of the largest specimens is 5.6 mm. \times 1.2 mm. The oral sucker is somewhat elongated. The oesophageal lumen is wide and its thickening is very remarkable. The bifurcation of the intestine lies at the anterior third. The intestinal caeca are almost straight. The anterior ends of the vitellaria are situated at the level of the anterior margin of the testes, not attaining the level of the oesophageal bifurcation. The egg is decidedly smaller than in other species of the genus, measuring 0.07–0.075 mm. \times 0.045 mm.

(15) **Pneumonoeces** sp.

This species, of which I have examined a few specimens only, was found in the lung of *Rana nigromaculata* collected in Tokyo. The cuticle is armed with spines.

(16) **Loxogenes liberum** Seno.

I found many examples of this species in the duodenum of *Rana nigromaculata* collected in Tokyo, etc.

IV. FISH TREMATODES.

(17) **Leptolecithum eurytremum** n. g., n. sp. (Pl. XXVI, fig. 1).

Leptolecithum n. g.

Diagnosis. Hemiuridae. Body moderately large, flat; widest at posterior third. Both suckers well developed; ventral sucker at anterior third of body length. Glandular stomach well developed; intestinal caeca with several lateral windings and terminating blindly near the posterior end. Stem of the excretory vesicle bifurcates near the middle of the body and both arms extend almost to the pharynx; stem and arms both make several lateral windings. Testes lie symmetrically, immediately posterior to the ventral sucker. Vesicula seminalis a convoluted canal lying anterior to the ventral sucker. Ovary vermicular in form, lying transversely near the posterior end

of the body and forming a large loop. Vitellaria consist of several long branches, which are again subdivided. Laurer's canal present; receptaculum seminis absent. Uterus very long, forming several convolutions between the two intestinal caeca. Eggs fairly large, numerous. Habitat: in the air-bladder of fishes.

Type species: *L. eurytremum* n. sp.

Leptolecithum eurytremum n. sp.

Diagnosis. Size of mature distome 13 mm. \times 7 mm. Both suckers large, the ventral sucker being the larger of the two. Intestinal caeca make about five lateral bends. Stem of the excretory vesicle a broad canal at its posterior part, but at the level of the shell-glands it narrows abruptly and forms lateral bends between the intestinal caeca; at the middle of body it bifurcates, both arms make similar bends and finally end near the pharynx. Margin of testes entire. Vitellaria consist of about four to five large branches, which divide several times. Ovary a long tube about 2.3 mm. in length, lying transversely and often contorted. Uterus occupies the space between the intestinal caeca and makes about three large transverse bends, each of which has fine undulations. Cirrus-pouch (?) a large ellipsoidal muscular mass, slightly protruding in the depression between the pharynx and the ventral sucker. Eggs 0.048–0.05 mm. \times 0.023–0.025 mm. Habitat: in the air-bladder of *Parasilurus asotus* and other fish.

DESCRIPTION. The mature specimens of this species are found in the air-bladder of *Parasilurus asotus* and *Pseudobagrus auranticus*, more commonly in the former. Several specimens ordinarily occur in one host. In the coelom of the above two species of fish and *Hypomesus olidus* and *Richardsonius hakuensis*, immature specimens are found. These immature specimens are commonly found near the anterior part of the coelom or in the vicinity of the anus. The parasite has been collected from various places in the Okayama Prefecture, Sahara (Chiba Prefecture), Kasumiga-ura (Ibaraki Prefecture) and Lake Biwa.

A mature specimen measures 13 mm. long and 7 mm. wide at the broadest part. The body is compressed dorso-ventrally, broadest at the middle or the posterior third of the body, and tapering anteriorly and posteriorly. It has on the whole a leaf-like shape. The ventral surface is often slightly concave. In the fresh state, it appears flesh pink, showing blackish colour, due to the intestinal contents, along the intestine. The living parasites move on slowly by the contraction and elongation of the body.

The cuticle is smooth and measures 0.04 mm. in thickness. The oral sucker has a diameter of 1.2 mm. and is situated on the antero-ventral end. The ventral sucker is slightly larger than the oral, being 1.4 mm. in diameter and is situated at the anterior third. The pharynx is directly continuous with the oral sucker and has an elongate form, the size being 0.85 mm. \times 0.5 mm. In the entire preparation the oesophagus cannot be detected, but in sections a

short oesophagus is recognisable postero-dorsal to the pharynx, which soon bifurcates into two intestinal caeca which turn laterally at right angles and for a certain distance show the same structure as the oesophagus, being lined by cuticle. This part corresponds to the crop of *Distomum ampullaceum* as described by von Buttel-Reepen¹. Ultimately, each caecum widens into a sac-like portion, the glandular stomach (Drüsenmagen of the German author), which has special epithelium; each cell of which bears long flagella-like appendages, longest in the posterior portion, and measuring 0.06 mm. in length. The epithelium of the glandular stomach is continuous with the ordinary epithelium of the intestine. Both the caeca turn posteriorly and run along the lateral body margins with characteristic windings, some five turns; and then terminate near the posterior end of the body, where each approaches to the median line. The first turn of the intestine occurs near the anterior margin of the ventral sucker; the remaining four turns are situated posterior to the ventral sucker. The first turn is most remarkable and in some cases the caeca almost meet in the median plane.

The excretory pore opens at the posterior end of the body. The vesicle has the form of an exceedingly elongate Y, the stem and the two arms of which bend laterally several times. In young specimens the stem is straight or slightly curved, while in mature specimens it bends at right angles at the level of the ovary to the right and forms a spindle-shaped broad tube, which becomes abruptly narrower and runs laterally and anteriorly, with two or three transverse bends, in between the intestinal caeca. Its anterior end reaches the middle part of the body and divides into two arms at the median plane. Each arm has a similar course to the stem, and after bending once or twice it reaches the level of the testes and passes over the intestinal caeca, then runs anteriorly along the lateral body margin and reaches as far as the pharynx. At this place it continues into the collecting vessels. The winding course is more marked in large and mature specimens, while in younger examples the entire vesicle usually takes a slightly undulating course.

Both the testes lie at the posterior lateral margin of the ventral sucker, immediately mesial to the intestinal caeca. Each of them has a somewhat round shape, and is 0.7 mm. in diameter. From their antero-mesial border the vasa efferentia arise. They run antero-medially and unite at the antero-dorsal margin of the ventral sucker to form the vas deferens. The vas deferens widens slightly to form the tube-like vesicula seminalis. In the mature specimen it bends 3-4 times, while in the younger form it remains straight. Anteriorly the vesicula seminalis enters into a cirrus-pouch-like muscular organ and continues into the ductus ejaculatorius. Both structures make several windings. The distal end of the ductus ejaculatorius is united with the vagina and forms the ductus hermaphroditicus. The cirrus-pouch-like organ is situated midway between the two suckers; it has an elliptical shape and measures

¹ Buttel-Reepen, H. von (1902). Zur Kenntniss der Gruppe des *Distomum clavatum*, etc. *Zool. Jahrb. Ab. Syst. etc.* xvii.

2 mm. \times 1.2 mm. At its anterior part a depression is present on the body surface, the genital atrium. A part of the cirrus-pouch-like organ protrudes into the atrium to form the genital papilla, at the apex of which the ductus hermaphroditicus opens.

The ovary lies at the level between the fifth bend of the intestinal caeca, slightly to the left of the median plane. It is tubular, winding irregularly several times; it measures about 2.3 mm. \times 0.15 mm. Its median end opens into a broad and short oviduct. Laurer's canal is present and opens dorsally, while the receptaculum seminis is lacking. The vitellaria are situated at the posterior part of the body around the ovary and between the intestinal caeca. The distal end of the glands passes over the intestinal caeca. They have a dendritic form, are finely branched, and, in the mature specimen, the left and right halves are barely distinguishable. The main branches are 5-6 in number; they are united with one another in the anterior median part and distally they divide into finer branches. The yolk duct arises at the anterior median part. It is short and soon forms a yolk reservoir. The shell-gland is diffuse and the glandular cells have long slender ducts. The uterus makes six loops and is situated between the two intestinal caeca; some parts pass over the caeca to the exterior and run at right angles to the longitudinal axis of the body. In each loop are found still smaller undulations, which are more numerous in the posterior portion. Around the uterus are certain glandular cells which have a similar appearance to the shell-glands, except that they are provided with a shorter duct. Distally the uterus runs directly over the dorsal border of the ventral sucker on the left side of the cirrus-pouch-like organ and is continuous with the vagina, which enters the cirrus-pouch-like organ. After making several windings it unites with the ductus ejaculatorius.

The eggs are numerous, the size being 0.048-0.05 mm. \times 0.023-0.025 mm. A distinct lid is present.

It is clearly seen that this species has affinities with the Hemiuridae, especially to the group of *Distomum clavatum*.

I have once found a very young specimen of this species in the gills of *Pseudobagrus auranticus*. The shape is ovoidal, the anterior end being rounded, while the posterior end is somewhat tapering. Its size is 0.35 mm. \times 0.2 mm.

The broadest part is the posterior third of the body. The oral sucker lies at the antero-ventral end, the diameter being 0.12 mm. The ventral sucker lies slightly posterior to the middle of the body, the diameter being 0.18 mm. Between the suckers the cirrus-pouch-like organ lies, touching the oral sucker anteriorly and the ventral sucker posteriorly. Both testes lie directly postero-ventrally to the ventral sucker; their shape is ellipsoidal, and each measures 0.05 mm. in diameter. The ovary lies slightly posterior to the testes, between which it is transversely elongated in the median plane; its length is 0.03 mm. The vitellaria are seen as an irregular cell-mass, lying posteriorly to the ovary.

It is very interesting to note that in the coelom of the host no mature

specimens are met with. Similar young specimens are often found in the air-bladder. It seems probable, therefore, that the coelom is not a favourable situation for the maturation of this species.

A cercaria form, which seems to belong to this species, I shall reserve for future description.

(18) **Distomum** sp. from *Thynnus thynnus*.

(A species near *Distomum clavatum*.)

This species infests the stomach of *Thynnus thynnus*. One somewhat immature specimen was obtained from a fish in the Tokyo market.

It is roughly cylindrical in shape, the widest part being situated near the posterior end of the body. Anteriorly it has an almost uniform breadth and terminates in a rounded extremity, while posteriorly it tapers rapidly. The size of a moderately contracted specimen is 25 mm. long and 5 mm. wide in the widest part. The oral sucker is situated at the anterior end of the body, the aperture pointing forwards. It is cup-shaped, measuring 1.1 mm. both in length and dorso-ventral diameter. The ventral sucker lies about 4 mm. posterior to the anterior end. It is protruded remarkably and even pedunculated. The shape is a deep cup-form, and the main axis of the sucker runs obliquely. The length of the sucker is 2 mm. and the aperture of the lumen is 1 mm. in longitudinal diameter. In fixed specimens the anterior part of the body is bent dorsally at the level of the ventral sucker.

The cuticle is very thick and unarmed. The pharynx lies directly posterior to the oral sucker. It is spherical, the diameter being 0.7 mm. The oesophagus curves dorsally, its length being about 0.6 mm., and from it two pairs of the intestinal caeca arise, the anterior and the posterior. The anterior ramus is short, ending near the dorsal part of the pharynx; the posterior ramus reaches almost to the posterior end of the body and has numerous mesial and lateral branchings. The lateral branches are further subdivided.

The excretory vesicle opens at the posterior end of the body. Its unpaired part runs forward for about 5 mm. and then divides into two (?) arms. The unpaired part lies between the two intestinal caeca, its wall following an uneven course, alternating with the branches of the intestine. The lateral arms run posteriorly and, when nearing the posterior end of the body, they turn anteriorly and reach the dorsal part of the oral sucker. It seems probable that they run back again posteriorly. In their course numerous slight undulations are found.

The two testes lie in the median line immediately posterior to the ventral sucker, the anterior one overlapping the posterior. Their shape is spheroidal, the diameter being about 0.5–0.6 mm. Anterior to the ventral sucker is the vesicula seminalis. It is a broad tube and runs along the median plane, winding laterally several times. The pars prostatica is a long winding tube which leads to the ductus ejaculatorius. There is no cirrus-pouch. The ductus ejaculatorius opens on the genital papilla which lies in the genital atrium.

The distal part of the ductus ejaculatorius and the distal part of the female duct are surrounded by several longitudinal parenchyma muscles, forming a cirrus-pouch-like organ.

The ovary lies posterior to the posterior testis. It is round and is smaller than the testes, its diameter being 0.35 mm. The well-developed shell-gland is situated posterior to the ovary. Laurer's canal opens dorsally. The receptaculum seminis is lacking. The vitellaria are long and slender, running along the posterior intestinal caeca. They extend from the level of ovary to within 8 mm. of the posterior end of the body. The loops of the uterus lie between the posterior intestinal caeca and extend backwards almost to the anterior end of the unpaired part of the excretory vesicle. There is no distinct vagina, the whole length of the uterine duct being provided with a cuticular wall. No perfectly formed eggs were found in the specimen under my observation.

(19) **Distomum** sp. from *Muraenesox cinereus*.

This species seems to belong to the Accacoeliinae, Hemiuridae, and may, perhaps, be new; but as I have only one damaged specimen I content myself with its description, and leave its species undetermined.

It measures 11 mm. in length and 5 mm. in breadth in the posterior part, and it narrows anteriorly, the anterior end being 2 mm. wide. Its general form is conical, showing a somewhat circular outline in transverse section. The posterior end terminates roundly.

The cuticle shows remarkable closely set transverse foldings. Subcutaneous muscles well developed in the anterior part, but posteriorly they become weaker.

The oral sucker lies subterminally, its length being 0.6 mm. The ventral sucker is situated about 1 mm. behind the oral, and is deeply invaginated. It is 1.2 mm. in its entire length.

The pharynx continues directly into the oral sucker, its length and breadth both measure 0.8 mm. The oesophagus is longer than the pharynx and makes a dorsal bend, and then bifurcates into intestinal caeca, which form several turns and terminate near the posterior end of the body. At the place of the bifurcation the oesophagus has two anterior diverticula, which end blindly at the level slightly anterior to the pharynx. The diverticula seem to be homologous to the anterior ramus of the intestine in species previously described.

The two testes lie slightly obliquely with respect to one another near the posterior border of the ventral sucker. They are slightly and irregularly sinuate; their lengths are respectively about 0.2 mm. and 0.25 mm. The vesicula seminalis lies at the anterior dorsal part of the ventral sucker; its outline is ellipsoidal and near its middle part a septum-like narrowing is present. The pars prostatica is well differentiated, the length being 1 mm. The wall of this part contains several nuclei which bulge into the lumen.

The main portion of the wall is occupied by the terminal part of the prostate

glands which protrude into the lumen and lie closely side by side. This phenomenon—primary epithelial cells being degenerated and secondary wall formed by union of the ducts of the prostate glands—seem to show the processes of formation and the nature of the cuticula of the body surface and other parts in like manner.

The ovary lies near the posterior end of the body. It is 1 mm. in width and has numerous lobes. Dorsally to the ovary the mass of the vitellarium lies; it is somewhat larger than the ovary and is divided into several lobules. Laurer's canal ends in a vesicular cell mass, similar to that of *Lecithochirium* sp. described later. The uterus is a broad canal which first runs near the ventral body surface, and later near the dorsal, with many windings. Near the anterior dorsal part of the ventral sucker it is continued into the vagina which unites with the ductus ejaculatorius and forms a canal-like genital sinus. The genital pore opens near the oral sucker.

The eggs are numerous, spherical in form, and 0.015 mm. in diameter.

The parasite was found in the stomach of *Muraenesox cinereus*, captured near Okayama (1914).

(20) **Lecithochirium** sp. (Pl. XXVI, fig. 2).

This species infests the stomach of *Muraenesox cinereus*. The length of the body proper is about 3.5 mm. and the appendix 2 mm. The breadth is 1 mm., being broadest in the posterior part of the body proper. The ventral sucker lies 1–1.5 mm. from the anterior end. The diameters of the oral and the ventral sucker are 0.4 mm. and 1.6–1.9 mm. respectively. The size of the egg is 0.021 mm. \times 0.01 mm.

This species seems to be new to science¹.

(21) **Brachyphallus** sp.

This species infests the stomach of *Scomber japonicus*. The size of the body is 1–1.7 mm. \times 0.35 mm. The diameters of the oral sucker and the ventral sucker are about the same, being 0.12 mm. The pharynx has a diameter of 0.065 mm. The egg is 0.024 mm. \times 0.012 mm.

(22) **Lecithocladium** sp.

This species infests the stomach of *Scomber japonicus*. The body-length is 6.5–7.5 mm. The oral and the ventral suckers have lengths 0.45–0.5 mm. and

¹ In the description of this and other genera, Juel, H. O. (1889), Beiträge zur Anatomie der Trematodengattung *Apoblema* (Dujard.), *Bih. Till. K. Svenska Vet.-Akad. Handl.* xv. afd. iv. No. 16; Lander (1904), The Anatomy of *Hemiurus crenatus*, etc. (*Bull. Mus. Comp. Zool. Harvard*, XLV.), and Looss (1908), Beiträge zur Systematik der Distomen. Zur Kenntnis der Familie Hemiuridae (*Zool. Jahrb. Abt. Syst.* etc. xxvi), note the presence of the receptaculum seminis, which has a peculiar structure. After my observation this so-called receptaculum seminis is nothing less than the blindly-ending Laurer's canal which is surrounded by a mass of vesicular cells. A diagrammatic figure of this part is given in Pl. XXV, fig. 6. A similar structure is met with in *Distomum* sp. from *Muraenesox cinereus*, *Brachyphallus* sp., *Lecithocladium* sp. and *Didymozoon* sp., according to my observations.

0.14–0.24 mm. respectively. The elongated pharynx measures 0.45–0.625 mm. \times 0.12–0.25 mm. There are no cervical papilla-like process or fringes. The size of the egg is 0.016 mm. \times 0.008 mm.

This species appears to be new.

Some of the specimens were parasitised by a sporozoan (Haplosporidia?). The parasites infest only the parenchyma cells of the host.

(23) **Didymozoon** sp. (Pl. XXVI, fig. 4).

This species infests the gill of *Scomber japonicus*. The cyst has an ellipsoidal form, measuring 2 mm. \times 1.2 mm. Two individuals are present in one cyst. Both are hermaphrodite and have the same structure. The body is composed of two parts, a narrow anterior and the greatly enlarged posterior part. The anterior part has a length of about 0.7 mm. and a breadth of 0.08 mm. The posterior part measures 1–1.3 mm. in length and 0.55 mm. in breadth. At the junction of the two parts, the posterior part protrudes in two lateral lobes which overlap the anterior part. As Odhner¹ has observed, its form is comparable to that of a mammalian stomach, the anterior part corresponding to the oesophagus.

The oral sucker lies at the anterior end of the body, being placed in a depression. It is pyriform, the length and the width at its widest part being 0.035 mm. and 0.02 mm. respectively. The pharynx is spherical, being 0.025 mm. in diameter. The prepharynx is lacking. The oesophagus is relatively slender; it is divided into two intestinal caeca about at the middle of the anterior part. The intestinal caeca extend almost to the posterior end and they widen in their posterior parts.

The testes, the ovary, the vitellarium and the uterus lie in the posterior portion of the body. They are all very long and make numerous loops. The oviduct arises from the middle of the ovary. The vitellarium has two main trunks which each divide at least twice. The vitellarium extends throughout the whole peripheral part of the posterior portion, while the central part is occupied by the uterus. The ovary lies near the base of the anterior part. There is a Laurer canal, which ends blindly, being surrounded by a group of peculiar cells. Near the basal portion of the anterior part, the uterine walls become abruptly thickened to form a vagina, which runs straight forwards and opens at the anterior end of the body, near the mouth. The eggs are very numerous. They are small, being 0.018 mm. \times 0.007–0.009 mm.

Two testes lie near the base of the anterior part of the body. Each of them makes a single loop. At the anterior end the vas efferens arises which soon unites with the vesicula seminalis. The vesicula seminalis is a thick tubular organ running anteriorly between the intestinal caeca and later along the oesophagus. There is a short ductus ejaculatorius which opens at the anterior end, uniting with the vagina.

¹ Odhner, T. (1907). Zur Anatomie der Didymozoon, etc. *Sartryck ur Zoologiska Studier illa gnade Professor T. Tullberg*.

This species resembles in external form *Didymozoon lampridis*, but I am uncertain as to its identity on account of the lack of literature.

(24) **Lepodora** sp. (Pl. XXVI, fig. 3).

This species infests the intestine of *Oncorhynchus masou*. The body is elongated and compressed dorso-ventrally. The broadest part of the body lies slightly posterior to the ventral sucker and anteriorly tapers rapidly, while posteriorly it narrows gradually. Dorso-ventrally the thickest part lies at the level slightly posterior to the ventral sucker. The size is 5–6 mm. in length and 1 mm. in the widest breadth and 0.8 mm. in thickness dorso-ventrally.

The oral sucker is situated at the anterior end of the body, and the ventral sucker at the anterior fourth of the body-length. The diameters of the oral and the ventral suckers are 0.28–0.35 mm. and 0.42–0.6 mm. respectively. Both suckers are round in outline. The cuticle is armed with large scale-like spines which are present all over the body.

The oral sucker is succeeded by the prepharynx, the length of which is 0.55 mm. The pharynx is large and well developed, its length being 0.28 mm. The oesophagus is very short and it rapidly bifurcates into the intestinal caeca. The intestinal caeca are broad and straight and end near the posterior end of the body.

The unpaired part of the excretory vesicle is long and straight. It runs along the ventral body wall and is divided into two short arms at the level of the anterior testis.

The two testes are large and elliptical, and lie between the intestinal caeca, one behind the other, at the middle and the posterior third of the body-length respectively, so closely along the intestine as to press the latter somewhat outward. The length of the anterior testis is 0.7 mm. and the posterior one 1 mm. They lie separated from each other by a short interval. A large and curved cirrus-pouch is present. It extends posteriorly beyond the ventral sucker. The genital opening lies directly anterior to the ventral sucker. The space of the posterior half of the cirrus-pouch is filled with the broad and convoluted seminal vesicle. There is a relatively short pars prostatica lying in the middle part of the pouch. The ductus ejaculatorius has an epithelial wall and opens into the genital sinus.

The ovary lies directly anterior to the anterior testis. It is spherical in shape, its size being 0.3 mm. There is no receptaculum seminis. Laurer's canal opens dorsally. The vitellaria lie along the lateral body margins in the posterior part of the body, partly overlapping the intestinal caeca dorsally and ventrally. The uterus is relatively short, forming several transverse loops between the ovary and the ventral sucker. The vagina is not well marked. At the posterior margin of the ventral sucker the uterus is continuous with a somewhat muscular canal, which has an epithelial wall. This canal, or rudimentary vagina, runs anteriorly along the dorsal border of the cirrus-pouch,

and opens into the genital sinus, which connects the genital ducts with the external genital opening. The eggs are not numerous. The size is 0.035-0.037 mm. \times 0.018 mm. and the shell is very thin.

This species differs from *Lepodora* Odhner, 1905¹, in certain characteristics, namely (1) that the genital opening lies directly anterior to the ventral sucker in the median plane; (2) that there is a relatively long genital sinus; and (3) that the receptaculum seminis is not present. In general structure, however, it closely resembles *Lepodora*, but certainly it differs from *Lepodora rachiaena*. It seems to be a new species of the Genus *Lepodora*.

(25) **Phyllodistomum folium** (Ofers.).

I found the parasite in the bladder of *Pseudobagrus auranticus* collected in Lake Biwa and Kasumigaura.

(26) **Exorchis oviformis** n.g., n. sp. (Pl. XXVI, figs. 5 and 6).

Exorchis n. g.

Diagnosis. Very small. Body oval to spherical or even broader than long. Anteriorly tapers slightly, posterior end somewhat truncated. Cuticle spinose. Ventral sucker lies anterior to the middle of the body and is smaller than the oral. Alimentary canal consists of the short prepharynx, the pharynx, the short oesophagus and the intestinal caeca, which terminate near the posterior end. Excretory vesicle Y-shaped, arms extend to near the point of the intestinal bifurcation. Genital pore lies directly anterior to the ventral sucker. No cirrus-pouch. Testes lie symmetrically, exterior to the intestinal caeca, at the level of the middle of body; margin entire. Vesicula seminalis large, constricted in the middle. Ovary lies slightly posterior to the ventral sucker, nearly on the median line; smaller than the testis and weakly lobate. Broad Laurer's canal present. No receptaculum seminis. Vitellaria in the anterior lateral region of body. Uterus with several loops in the posterior half of the body, partly extending outside the intestinal caeca. Eggs medium sized; shell thin. Habitat: in the intestine of fishes.

Type species: *E. oviformis* n. sp.

Exorchis oviformis n. sp.

Diagnosis. Size 0.2-3 mm. \times 0.2-0.28 mm. Shape often spherical. Oral sucker lies at the anterior end, 0.055-0.077 mm. in transverse diameter. Ventral sucker 0.04 mm. in diameter, situated at the level of the anterior third. Two eye-spots are present in the dorso-lateral region of the oral sucker. Other median pigment spots often present. Pharynx 0.03 mm. in breadth. Oesophagus 0.01-0.015 mm. in length. Intestinal caeca end near the posterior body end. Testes round or ellipsoid. Vesicula seminalis very large. Ovary with four or five lobes. Follicles of vitellaria relatively few in number. Laurer's

¹ Odhner, T. (1905). Die Trematoden des arctischen Gebietes. *Fauna Arctica*.

canal broad and terminating blindly (?) near the dorsal surface. Uterus with several irregular loops lying within and without the intestinal caeca, in the posterior half of body. Eggs 0.04 mm. \times 0.02 mm. Habitat: in the intestine of *Parasilurus asotus*.

DESCRIPTION. This parasite is found abundantly in the duodenum and the anterior part of the small intestine of *Parasilurus asotus*. The species occurs very commonly in the fish from various parts of Okayama and Tokyo.

Various stages of the species are found in one host. The average size of mature examples is 0.2–0.3 mm. in length and 0.26–0.28 mm. in breadth¹. Generally the body is slightly broader than long. Both anterior and posterior ends are round, the anterior part often narrowing slightly. It is compressed dorso-ventrally. It appears as a brown dot to the naked eye, the colour being derived from the eggs in the uterus.

The cuticle is relatively thick, measuring 0.003–0.004 mm. In fresh specimens closely-set, fine spines are found in the cuticle, excepting on the posterior part of the body. In sections, however, these spines appear indistinct, being represented as small protuberances on the surface. Several cephalic glands lie in the anterior end of the body near the oral sucker.

The oral sucker is situated at the anterior end, facing ventrally. In moderately extended specimens, the ventral sucker lies at the anterior third of the body and it is smaller than the oral one, the diameters of the oral and the ventral suckers being 0.055–0.077 mm. and 0.04 mm. respectively; the ventral is deeply depressed.

A pair of round, brown eye-spots are present at the anterior dorsal part of the body, laterally to the oral sucker. There is another median pigment spot lying slightly anterior or posterior to the paired eye-spots. In mature specimens they are indistinct and in some cases they are found disintegrating into several pigment granules.

The pharynx is small. Its breadth is 0.03 mm. The intestinal caeca turn first laterally and then posteriorly along the lateral body margin and terminate near the posterior end of the body, where they turn mesially.

The excretory vesicle is V-shaped, opening at the posterior end of the body. The arms are very broad and lie between the intestinal caeca and anteriorly they reach a point immediately posterior to the oesophagus.

Both testes lie near the middle part of the body on the same level, either on the dorsal side of, or lateral to, the intestine as the case may be. The shape is round or elliptical. The vesicula seminalis is very large and lies dorsally to the ventral sucker. It is 0.15 mm. \times 0.05 mm. and its dorso-posterior end almost touches the dorsal body wall. Slightly distal to the middle part, the organ is constricted, the constriction dividing the organ into two unequal parts. Antero-ventrally it narrows into a short ductus ejaculatorius which unites with the vagina and forms a tube-like genital sinus. It opens at the anterior border of the ventral sucker. It is destitute of a cirrus-

¹ As measured in a preserved specimen.

pouch, and no distinctly differentiated pars prostatica is present, excepting a few glandular cells lying around the proximal part of the ductus ejaculatorius.

The ovary lies posterior to the ventral sucker. It is smaller than the testes and has 4-5 lobes. The vitellaria occupy the anterior dorsal part of the body and have a dendritic outline. It is doubtful whether Laurer's canal has an external opening or not; the canal is a relatively broad tube which runs dorsally, side by side with the wall of the vesicula seminalis, and seems to end blindly. The uterus lies surrounded by the intestinal caeca. It forms several loops posterior to the ventral sucker, and some of them go exteriorly beyond the testes. The vagina, relatively long and slender, runs across the ventral sucker and ultimately unites with the ductus ejaculatorius. The egg measures 0.04 mm. \times 0.02 mm. and has a distinct lid. The shell is brown and relatively thick. In the distal part of the uterus, eggs are found containing embryos in an advanced stage of development.

I have often found encysted distomes in fresh water fishes, which bear a close resemblance to *Exorchis oviformis*, differing only from the latter in the absence of eggs in the uterus of the former. Young specimens of *Exorchis oviformis* are often observed in the intestine of *Parasilurus asotus*. No eggs are present in such specimens, while they have the same structure as the encysted distomes above mentioned. As the host of *Exorchis oviformis* usually feed on smaller fresh-water fishes, it is probable that this encysted distome is the young stage of the above parasite.

This encysted distome is found in various species of fishes; especially in *Pseudorasbora parva*, various species of *Leucogobio*, various species of *Acheilognathus*, *Carassius auratus*, *Richardsonius hakunensis*, various species of *Zacco*, and *Misgrunus anguillicaudatus*.

The encysted distome lies in the muscles, under the scales or in the fins. The cyst is spherical with a somewhat irregular shape, the diameter being 0.1-0.2 mm. The wall of the cyst is hyaline and structureless. Removed from the cyst, the worm (Pl. XXVI, fig. 6) has an ovoid outline, narrowing anteriorly, while posteriorly it terminates roundly. In the cyst, the worm is curved on itself or shortened lengthwise. The skin is closely beset with fine spines, excepting the posterior part of the body. These spines can be detected only in the fresh state. The oral sucker occupies the anterior end and the ventral sucker is situated slightly anterior to the middle of the body-length. The latter is smaller than the former, the diameters being 0.05 mm. and 0.035 mm. respectively. The parenchyma is transparent. In the anterior dorsal part of the body one pair of eye-spots and one median pigment spot are present. The eye-spots are situated symmetrically on either side of the oral sucker, with the median pigment spot between them. The median spot is often excentric, and in some cases it is wanting entirely.

Posterior to the oral sucker is a small pharynx measuring 0.023 mm. \times 0.02 mm. and this is continuous posterior with the short oesophagus which divides into intestinal caeca; these turn outwards and backwards along the

lateral body margin and reach almost to the posterior end of the body. The excretory vesicle is V-shaped and lies between the intestinal caeca and opens at the posterior end of the body. The lumen of the vesicle contains refractile excretion granules. At the middle of the body and laterally or dorsally to the intestine are the testes. They lie at the same level. Directly posterior to the ventral sucker is a small cell-mass which represents the ovary.

Exorchis oviformis seem to have a close affinity with *Stegopa globosa* Linton, which was discovered in the intestine of a marine fish by Linton¹. The description and the illustration of *Stegopa* are very incomplete, so I cannot identify them. I may add that there is little affinity between the two genera *Siphodera* and *Stegopa*, which are comprised in one family, Siphoderidae, by Linton.

(27) ***Steringotrema nakazawai*** n. sp. (Pl. XXV, fig. 7).

Diagnosis. Body small, 2.3–3 mm. \times 2–2.8 mm.; posterior half broadest. Ventral sucker 1 mm. \times 1.5 mm. Alimentary canal consists of a spherical pharynx, short oesophagus and narrow intestinal caeca which run along the lateral margins and end near the posterior end of the body, approaching each other at their distal extremities. Excretory vesicle V-shaped, arms reaching almost to the oral sucker. Testes entire, round, 0.3–0.4 mm. in breadth. Ovary with several lobes, transversely elongated, 0.2 mm. in breadth. Vitellaria consist each of from 5 to 6 groups. Loops of uterus run along the posterior and left side of the ventral sucker. Eggs 0.045 mm. \times 0.025 mm. Habitat: in the intestine of *Sparus latus*.

DESCRIPTION. This species infests the intestine of *Sparus latus*, and the specimens were collected by K. Nakazawa at Taka-no-Shima, Awa Province, in 1911.

Body measures 2.3–3 mm. \times 2–2.8 mm. The shape is somewhat ovoidal, the posterior half being broadest, and anteriorly it gradually tapers, while posteriorly it ends roundly. The ventral surface is ordinarily flat or concave, while the dorsal surface is somewhat convex. In alcohol the specimen is pale blue.

The cuticle is smooth and relatively thick, being 0.005 mm. The oral sucker is situated at the anterior end of the body, and turned towards the ventral surface, the diameter being 0.1–0.3 mm. The ventral sucker is very large, occupying the main part of the posterior broad part of the body, it is broader than long, measuring 1 mm. \times 1.5 mm.

The oral sucker is directly connected posteriorly with the pharynx, without the intervention of a prepharynx. The pharynx is 0.2 mm. \times 0.2 mm. A short oesophagus is present. Its length is 0.2 mm. The intestinal caeca run along the borders of the ventral sucker, as far as the posterior border of the latter, and end near the median line. The lumen of the intestine is relatively narrow.

Excretory vesicle V-shaped, and opening on the dorsal surface near the

¹ Linton, E. (1910). Helminth fauna of the Dry Tortugas. II. Trematodes. *Publication No. 133, Carnegie Institution.*

posterior end of the body. Both arms run along the inner side of the intestinal caeca. Anteriorly they almost reach the posterior lateral border of the oral sucker.

Both the testes lie at the same level between the postero-lateral border of the ventral sucker and the intestinal caeca. They have a round or ellipsoidal shape, being 0.3–0.4 mm. in diameter. There is a well-developed cirrus-pouch, which is situated between the anterior part of the two intestinal caeca and the anterior border of the ventral sucker and slightly to the left of the median plane. It is somewhat round, the diameter being 0.5 mm. In the cirrus-pouch is the vesicula seminalis and the pars prostatica, the latter being divided into two parts by a constriction; the proximal part is narrower and curved, while the distal part is broader and short. The pars prostatica is long and wide and follows a circular course. In the genital sinus and near the opening of the pars prostatica is an invagination, which seems to perform the function of penis. There is no distinctly differentiated ductus ejaculatorius in the distal part of the pars prostatica, for the latter opens directly into the genital sinus. Around the pars prostatica are numerous prostate glands which fill up the entire space of the cirrus-pouch. The ducts of the glands terminate as long cilium-like processes on the wall of the pars prostatica. The processes lie closely together, their length being 0.02–0.06 mm.

The ovary lies on the posterior median side of the right testis, near the posterior margin of the ventral sucker, and a little to the right of the median plane. It has a somewhat lobate form, being broader transversely. It is smaller than the testis, the transverse diameter being 0.2 mm. The oviduct arises from the median border of the ovary. The vitellaria lie exterior to the intestinal caeca extending along the side of the ventral sucker. Each vitellarium consists of 5–6 ascini, each of which has a special yolk duct which unites with the rest to form a main duct. The main yolk duct runs mesially and unites with its partner from the opposite side in the median plane near the posterior margin of the ventral sucker, and forms a common duct which soon widens into a yolk reservoir and then opens into the oviduct. Laurer's canal is present, and takes a winding course in its proximal part, but finally it runs straight and opens to the dorsal surface. The receptaculum seminis is totally wanting. The loops of the uterus lie side by side with the posterior and left-hand border of the ventral sucker. At the side of the cirrus-pouch it is continuous with the vagina. The female genital opening lies close to that of the cirrus-pouch, in the genital sinus. The proximal part of the uterus widens slightly. The spermatozoa fill up this portion.

The genital sinus lies slightly to the left of the median plane, towards the ventral and anterior margin of the cirrus-pouch. It is nothing more than a depression of the body surface, provided with double circular folds.

The size of the egg is 0.045 mm. \times 0.025 mm. The shell is relatively thick and the lid is conspicuous. The eggs in the distal part of the uterus contain embryos at a quite advanced stage of development.

The genus *Steringotrema* Odhner, 1911, has a close affinity with *Didymorchis* Linton¹, and they seem to be identical.

I have in my possession several other species of Digenea, but their examination is incomplete; the publication of their descriptions must be postponed.

EXPLANATION OF PLATES XXIV—XXVI.

PLATE XXIV.

Fig. 1. *Microtrema truncatum* n. g., n. sp. $\times 10$.

Fig. 2. *Eurytrema pancreaticum* (Janson). $\times 5$.

Fig. 3. *Eurytrema coelomaticum* (Giard and Billet). $\times 5$.

In Figs. 2 and 3 merely the outline of suckers and gonads are given.

Fig. 4. *Eurytrema satoi* n. sp. $\times 18$.

Fig. 5. *Dicrocoelium macaci* n. sp. $\times 20$.

Figs. 6–8. Ditto, showing various shapes and situations of the body and the gonads. $\times 10$.

Fig. 9. *Cricocephalus koidzumii* n. sp. $\times 14$.

PLATE XXV.

Fig. 1. *Watsonius macaci* n. sp. $\times 5$.

Fig. 2. *Fasciolopsis buski* (Lank.), showing excretory vesicle. $\times 3$.

Fig. 3. Ditto; longitudinal section of the skin and the underlying tissues of the ventral part of body. $\times 170$.

Fig. 4. *Polyangium miyajimai* n. sp. $\times 10$.

Fig. 5. *Octangium takanoi* n. sp. The uterus is not figured. $\times 10$.

Fig. 6. Diagrammatic illustration of the Laurer's canal, the ootype and their vicinity of *Lecithochirium* sp. $\times 120$.

Fig. 7. *Steringotrema nagazawai* n. sp.

PLATE XXVI.

Fig. 1. *Leptolecithum eurytremum* n. g., n. sp. $\times 10$.

Fig. 2. *Lecithochirium* sp. $\times 10$.

Fig. 3. *Lepodora* sp. $\times 20$.

Fig. 4. *Didymozoon* sp. $\times 40$.

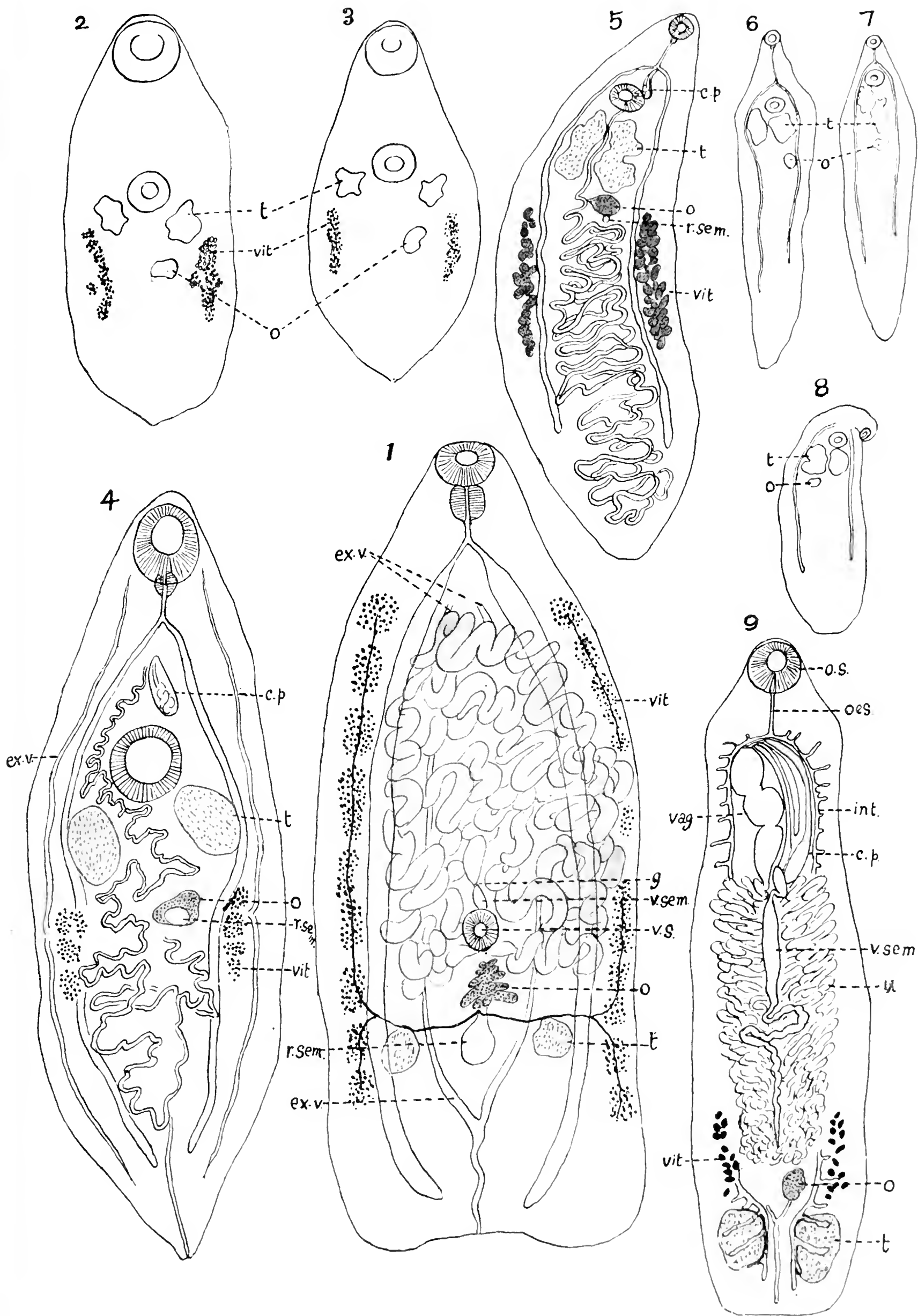
Fig. 5. *Exorchis oviformis* n. g., n. sp. $\times 200$.

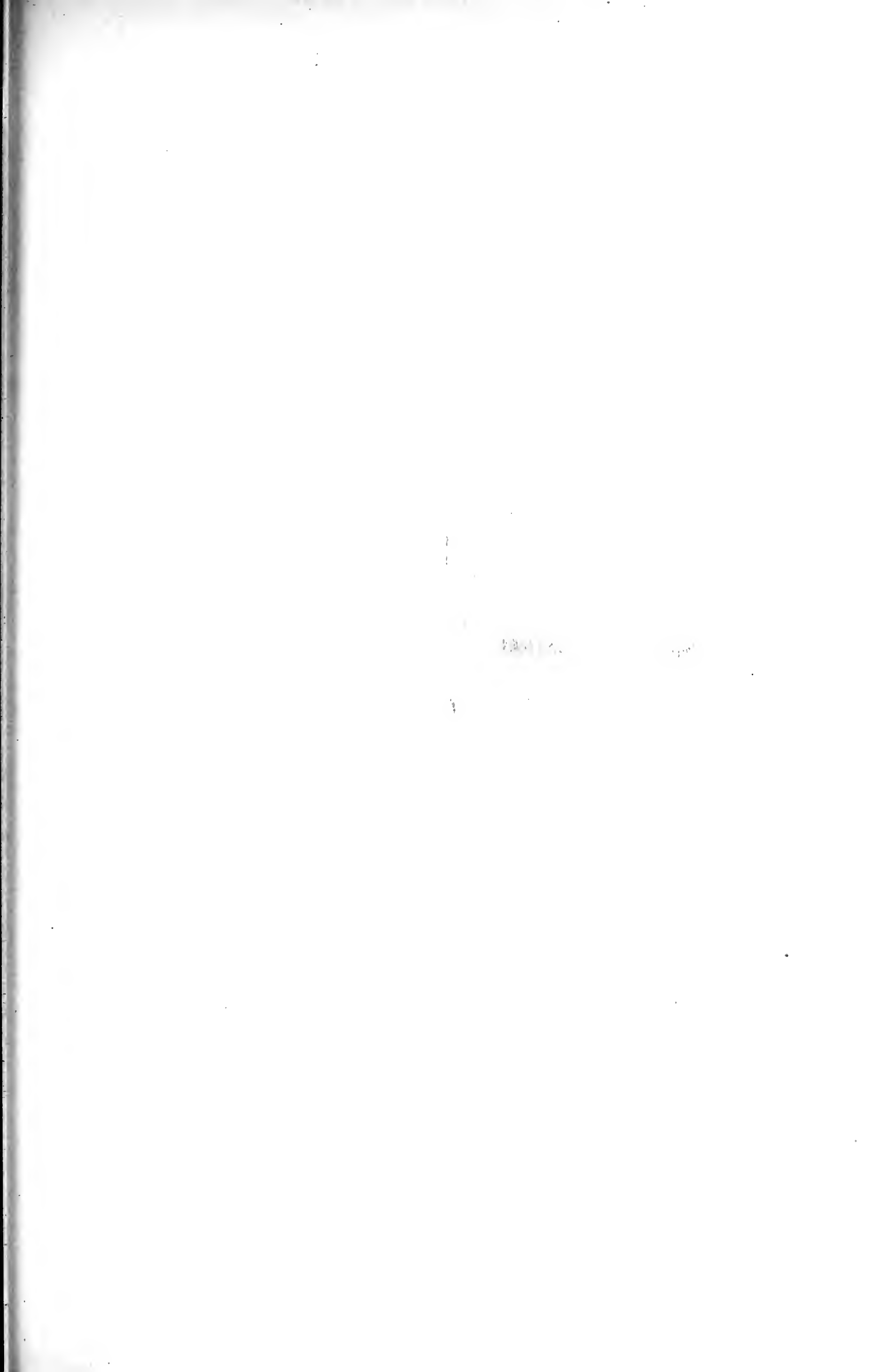
Fig. 6. Ditto; young, encysted worm. $\times 40$.

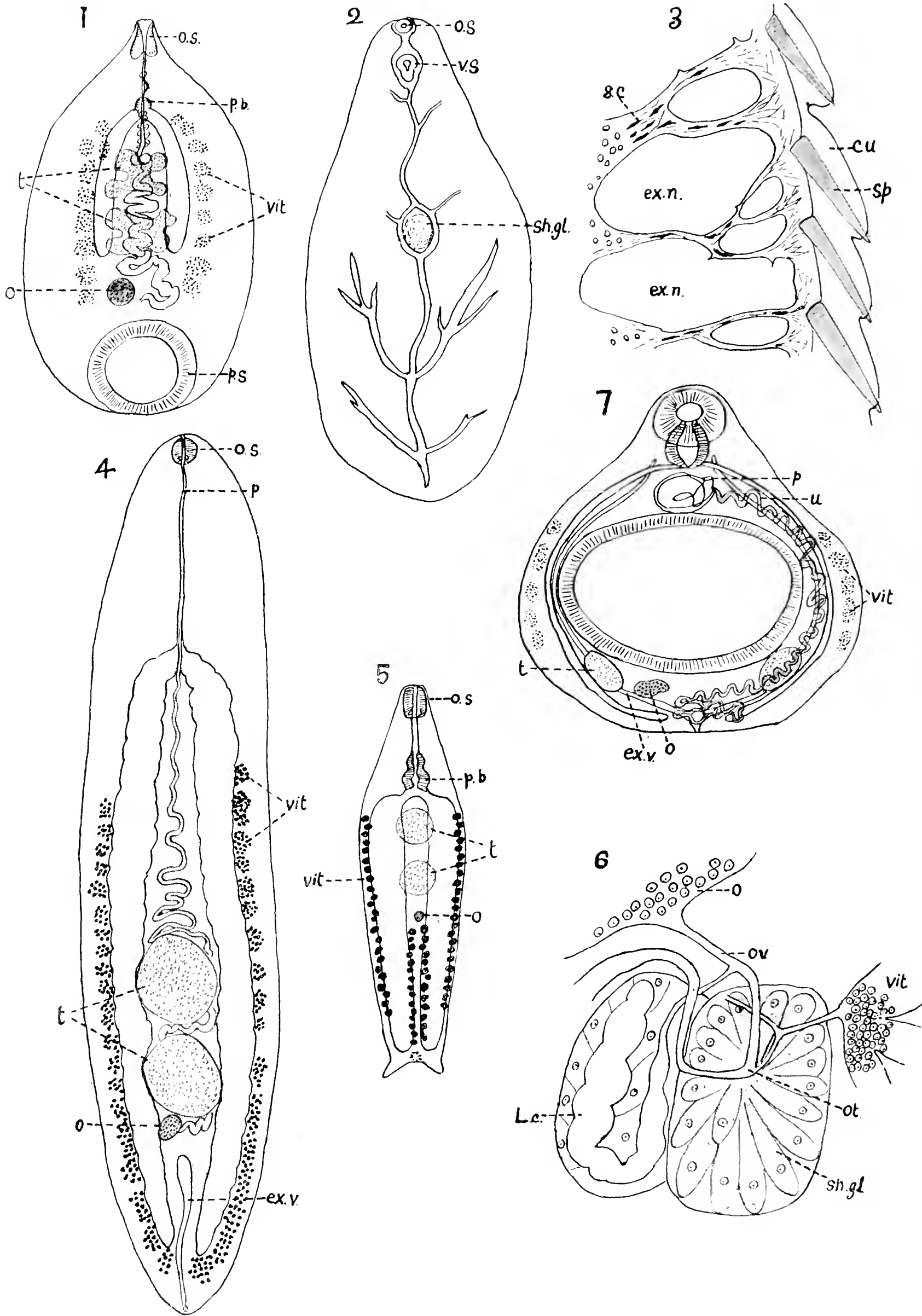
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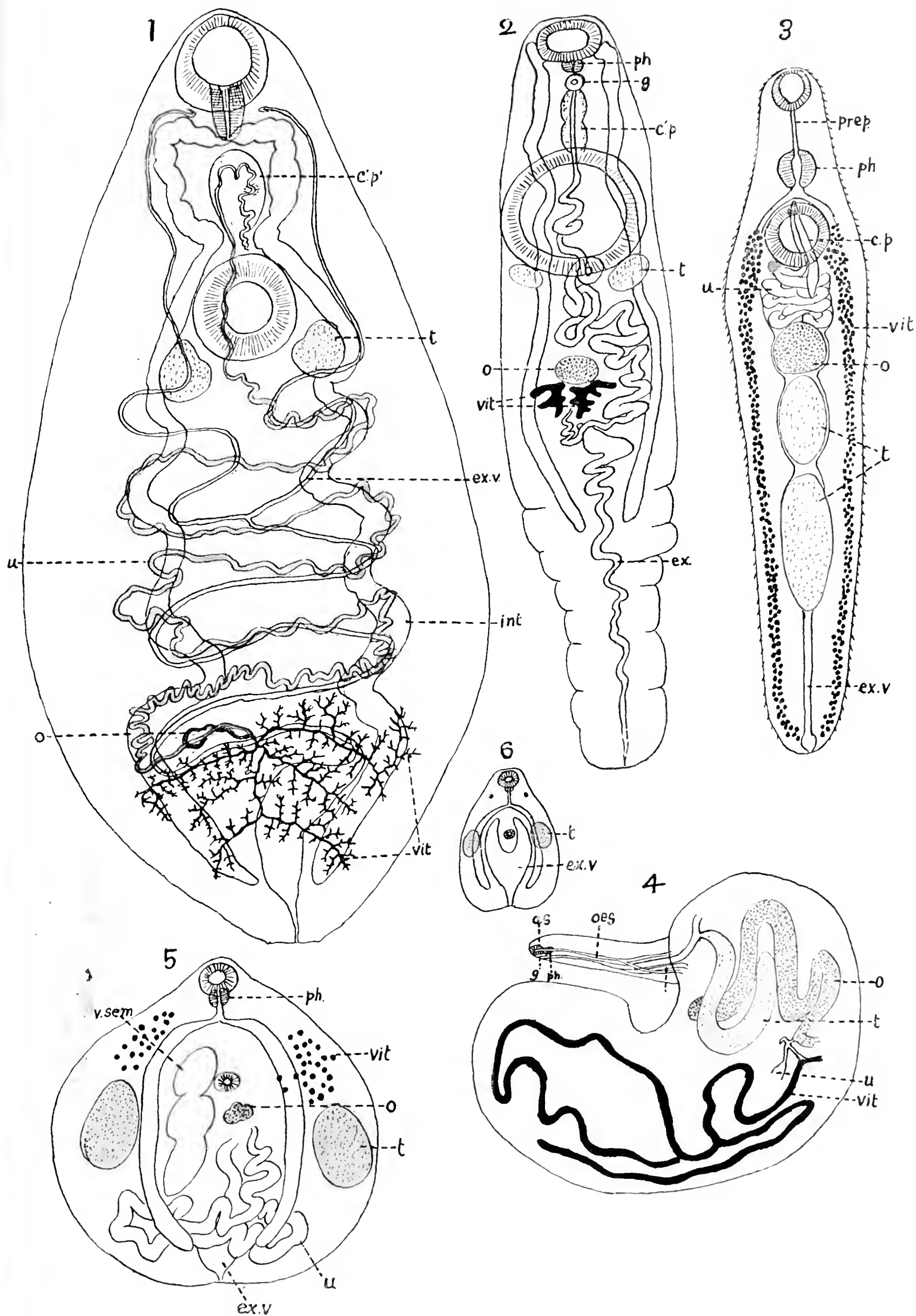
c.p. = cirrus-pouch; *c'p'* = cirrus-pouch-like organ; *cu.* = cuticula; *d. ej.* = ductus ejaculatorius; *ex. n.* = peripheral network of the excretory vesicle; *ex. v.* = excretory vesicle; *g.* = genital pore; *int.* = intestine; *L. c.* = Laurer's canal; *o.* = ovary; *o. s.* = oral sucker; *oes.* = oesophagus; *ot.* = ootype; *ovd.* = oviduct; *p. b.* = pharyngeal bulb, or muscular part of the oesophagus; *p. s.* = posterior sucker; *ph.* = pharynx; *prep.* = prepharynx; *r. sem.* = receptaculum seminis; *s. c.* = subcuticular cells; *sh. gl.* = shell gland; *sp.* = spine; *t.* = testis; *u.* = uterus; *v. sem.* = vesicula seminalis; *vag.* = vagina; *vit.* = vitellarium.

¹ Linton, E. (1910), *l.c.*









ON THE CLASSIFICATION OF THE ASCARIDAE.

II. THE *POLYDELPHIS* GROUP; WITH SOME ACCOUNT OF OTHER ASCARIDS PARASITIC IN SNAKES.

By H. A. BAYLIS, M.A.

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(With 7 Text-figures.)

THE Ascarids parasitic in snakes present considerable difficulties from the systematic point of view, owing to the incomplete character of many of the descriptions, and to the state of confusion which has long existed in the nomenclature. The writer has made an attempt, by examining such material as was available, and by comparing a number of the existing descriptions, to clear up some of this confusion. It was hoped also that such a comparative treatment would throw some light on the systematic value of the modifications of the female genital apparatus, which occur in some of these forms.

The typical female *Ascaris*, as is well known, possesses two uterine branches, commonly running backwards and parallel to each other from their point of origin in the common uterine tube. Retzius, in 1830, was the first to describe a multiplication of the uterine branches, occurring in an Ascarid from the python, subsequently redescribed and named *Ascaris anoura* by Dujardin (1845). In this form the uterus is divided into four, instead of two, branches, and Dujardin regarded it as representing a sub-genus, which he named *Polydelphis*, of the genus *Ascaris*. Since that time other forms having a four-branched uterus have been described from snakes. It was not, therefore, very surprising to the writer to discover, in 1916, an Ascarid from a snake showing a still further increase in the number of uterine branches. This was *A. boddaertii* Baird, in which the branches were found, on re-examination of the type, to be six in number. About the same time Geddoelst (1916) described a form, *Ascaris hexametra*, from a chamaeleon, in which a similar structure was seen; and more recently the writer has observed the same modification in two more Ascarids from snakes.

The Ascarids of snakes seem, as far as can be ascertained at present, all to belong to the sub-family Ascarinae. They show remarkably few characters which can be regarded as of more than specific value, in the present state of our knowledge of the Ascarid family as a whole. Most of them, however, appear to fall into two fairly well-marked groups, which may, provisionally at least, be treated as genera.

The first group consists of forms in which the uterus is of the primitive two-branched type, but with the vulva usually situated in the posterior region of the body, and rarely in front of the middle, its more usual position among the *Ascarinae*. These forms are further characterised by the presence of more or less well-developed interlabia between the main lips, and usually by having the cuticle at the bases of the main lips deeply grooved by an incision running in from the interlabium on either side.

The second group consists of species in which the uterus breaks up into more than two (four or six) branches, the vulva is situated, with few exceptions, in front of the middle of the body, and interlabia are absent. One species not from a snake, but from a chamaeleon, is included in this group.

Among the characters common to all, or most, of the species comprised in both groups are the general form of the spicules of the male, which have a tubular shaft and two broad membranous alae; the presence of simple dentigerous ridges on the inner surfaces of the lips; and the character of the ova, which are in all cases large, nearly spherical or of a much rounded oval shape, with a thick shell ornamented with granulations externally, and with the contents only segmenting at the time of laying. A further character which is not improbably universal is the presence of series of radiating digitiform processes on the anterior borders of the pulps of the lips. These processes are sometimes obvious in some specimens and hard to detect in others of the same species, and their visibility or otherwise evidently depends to a large extent on the state of preservation of the material and the technique employed in its examination. It is difficult, therefore, to make use of this character for systematic purposes.

The two main groups already referred to, and their component species, may now be diagnosed.

(1) **Ophidascaris**, n.g.

GENERIC DIAGNOSIS. *Ascarinae*: Lips almost square, with more or less rounded angles, and generally as broad as long. Dorsal lip slightly smaller than ventro-lateral lips. Interlabia usually well-developed. From the interlabia deep transverse grooves in the cuticle run partially round the bases of the main lips towards their main axes. Oesophagus relatively short, without bulb or ventriculus. No oesophageal or intestinal caeca (the intestine, however, is frequently pushed up into an annular "caecum" round the posterior end of the oesophagus). Vagina and uterus run backwards from the vulva. Uterus with two parallel branches. Vulva usually behind middle of body, and genital organs (in both sexes) usually confined to the posterior region of the body, which often shows a fusiform thickening.

GENOTYPE: *O. filaria* (Dujardin, 1845).

1. *Ophidascaris filaria* (Dujardin, 1845).*Ascaris filaria* Dujardin (1845), pp. 177, 653.

,, ,, Stossich (1896), p. 79.

,, ,, Railliet and Henry (1910).

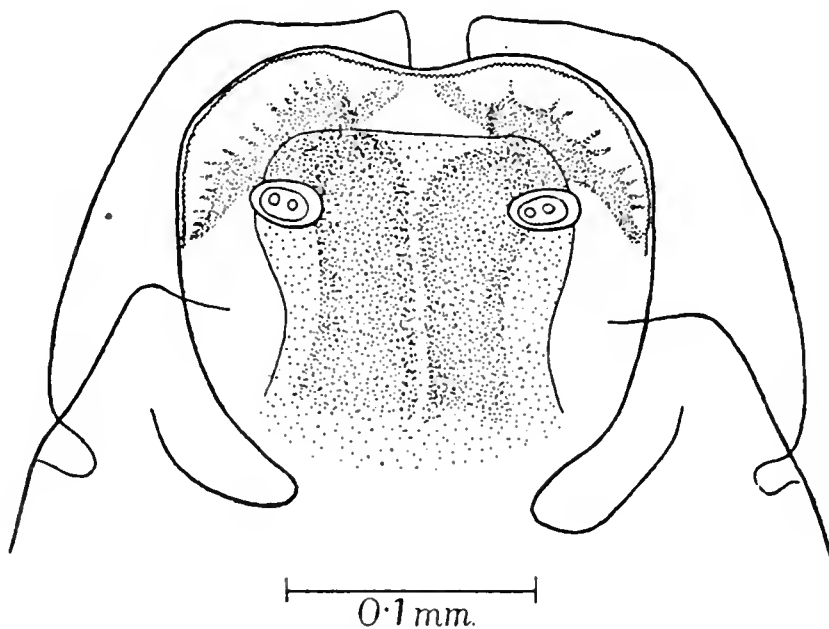
,, *rubicunda*, ♂, Schneider (1866), p. 42; Text-fig.; Pl. I, fig. 8.

,, ,, Stossich (1895), p. 35; Pl. VI, figs. 30, 31, 35.

,, ,, ,, (1896), p. 35.

? ,, *infundibulicola* v. Linstow (1903), p. 108; Pl. V, figs. 1-2.

Length, male, up to 110 mm.; female up to 170 mm. Thickness, male, 1 mm.; female, 1.5 mm. Cuticular striations about 3μ apart. Body cylindrical, greatly elongate, a little more tapering in front than behind. Lips (fig. 1) each bearing two papillae externally; the papillae of the dorsal lip with double terminations; ventro-lateral lips each with a large papilla towards the ventral and a smaller papilla towards the dorsal side. Dorsal lip with slightly emarginate

Fig. 1. *Ophidascaris filaria*. The head; dorsal view.

anterior border and rounded free angles. Free end of each lobe of pulp multi-radiate, with an antler-like lobule directed laterally and posteriorly. Interlabia short, bluntly conical. Well-developed grooves at bases of lips. Marginal dentigerous ridges present. Oesophagus 5-7 mm. long, somewhat swollen posteriorly. Excretory pore at about 1.4 mm. from the anterior end. Tail of male bluntly conical. Spicules slightly unequal, rounded at the tip, measuring about 4-4.8 mm. in length. Width of spicules, including shaft and alae, 0.06-0.07 mm. Postanal caudal papillae six pairs, of which pairs 1-5 form an almost circular group on either side near the tip of the tail, the sixth pair being situated a little behind the cloaca, and having double terminations. Tail of female bluntly conical, about 0.3 mm. long. Vulva situated considerably behind middle of body, dividing the total length in the proportion of about

7 : 4¹. Vagina simple, muscular, 0.15 mm. in diameter, sometimes running forward at first from the vulva, then doubling back with a sinuous course, and passing, a little behind the level of the vulva, into an oval swelling (measuring about 0.4×0.27 mm.). This is followed by a straight, unpaired portion of the uterus, about 4 mm. long, and with a maximum width of 0.4 mm. The uterus divides into two wide branches, which run posteriorly, parallel to each other, forming a few bends at first, and then becoming quite straight. Posteriorly these suddenly pass into two short, narrow, muscular ducts; which, after a course of about 0.7 mm., expand into fusiform swellings and then join the oviducts. The ovaries turn forward soon after their origin, the anterior limit of their coils being at about the level of the bifurcation of the uterus, from which point they return to the posterior end, and terminate at about 1.5 mm. in front of the anus. Ova nearly spherical, measuring 0.065–0.073 mm. in diameter.

HOSTS: *Python molurus*, *P. reticulatus*, *P. sebae*, *P. spilotes*; also *Varanus* sp. (Zanzibar—in British Museum collection).

GEOGRAPHICAL RANGE: Africa, India, Malay Peninsula and Archipelago, Australasia.

2. *Ophidascaris radiosa* (Schneider, 1866).

Ascaris radiosa Schneider (1866), p. 42; Text-fig.; Pl. I, fig. 9.

„ „ Stossich (1896), p. 36.

Length, male, 160 mm.; female, up to 270 mm. Maximum thickness 1.5 mm. The British Museum collection contains a single female specimen, 107 mm. long and 0.85 mm. thick, which is probably referable to this species. As Schneider did not describe the female beyond giving its size, this specimen will help to complete the description. It very closely resembles *O. filaria*. The lips, however (Fig. 2), show an important difference in outline. The dorsal lip is nearly square, with the anterior border more strongly emarginate than is the case in *O. filaria*, and the free angles much more acute. The ventral angles of the ventro-lateral lips project in a striking manner. The cephalic papillae are similar to those of *O. filaria*. The dentigerous ridges have not been seen (according to Schneider, they are present at some distance from the edge of the lip). The lobes of the pulp are multiradiate. Interlabia short. Well-marked grooves at bases of lips. Oesophagus about 4 mm. long. Tail 0.2 mm. long, ending in a short spike. Vulva situated within the posterior third of the body, dividing the total length in the proportion of 15 : 6.5. The vagina runs straight back, and the uterus has two wide branches. The whole of the female organs lie in the region between vulva and anus. Ova spherical, measuring 0.08 mm. in diameter.

The male, according to Schneider, has a slight caudal “bursa,” two pairs of almost lateral postanal papillae near the tip of the tail, and a single row of

¹ Railliet and Henry (1910) give “ $\frac{4}{7}$ ” from the posterior end, probably a *lapsus* for $\frac{4}{11}$. v. Linstow, for *A. infundibulicola*, states, probably by a clerical error, that the vulva is in front of the middle, dividing the body in the proportion of 4 : 7.

six preanal papillae on either side, passing anteriorly into a double row, "probably again becoming single."

Host: *Bitis gabonica*.

DISTRIBUTION: Africa.

3. *Ophidascaris obconica* (Baird, 1860).

Ascaris obconica Baird (1860), p. 447.

" " Baird (1861), p. 229.

" " Örley (1882), p. 310.

" " Stossich (1896), p. 80.

" " Baylis (1916), p. 413; Text-figs. 1-3.

This species has already been fairly fully re-described by the writer (1916). The following is a short summary of its chief characters.

Length up to 52 mm. (female); males slightly smaller. Maximum thickness 2 mm. A fusiform thickening of the posterior region of the body. Cuticular

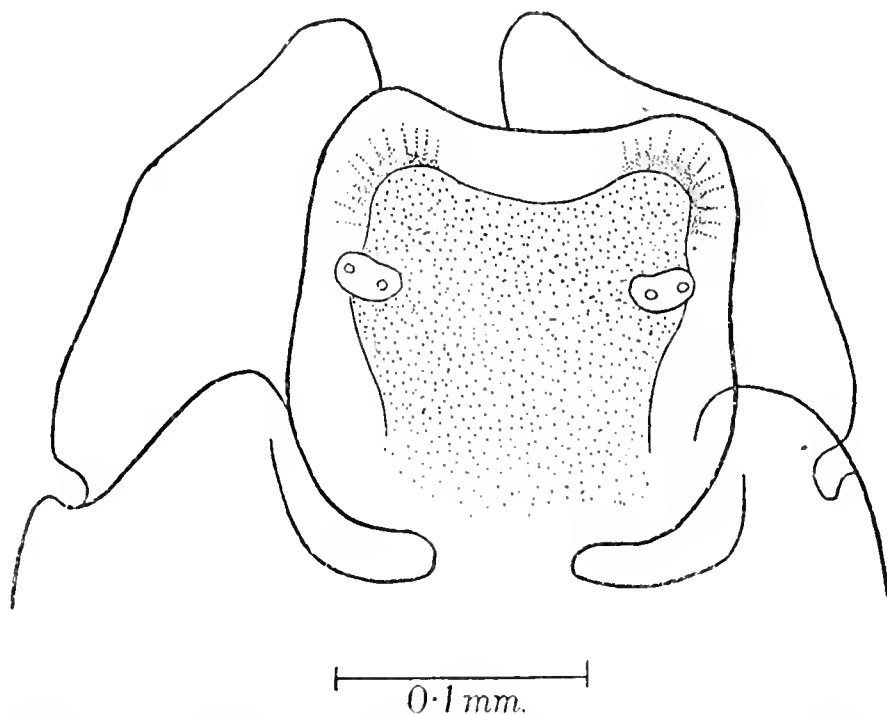


Fig. 2. *Ophidascaris radiosa*. The head; dorsal view.

striations 4.2μ apart. Lips almost square, with an indentation in the anterior margin, and with the free angles rounded. Dentigerous ridges marginal. Interlabia small, conical. Well-marked grooves at bases of lips, almost meeting in middle line of lip. Oesophagus 2-3 mm. long. Tail in both sexes very blunt, without terminal spike. Spicules of male 2.48 mm. long and 0.08 mm. wide. Four pairs of postanal papillae, near tip of tail, and about 40 pairs of preanal papillae. Vulva in posterior third of body, dividing the total length in about the proportion of 12 : 5. Vagina runs forward, passing into the uterus a little in front of the vulva; uterus then bends back and gives off two branches which run, with many convolutions, parallel to each other towards the posterior end. Ova spherical, 0.1 mm. in diameter, with coarsely granulated shell.

Host: *Helicops angulatus*.

LOCALITY: Brazil.

4. *Ophidascaris mombasica*, sp.n.

Length, female, up to 100 mm. (The only male available is not quite complete.) Maximum thickness, male, 1.7 mm.; female, about 2 mm. Cuticular striations exceedingly fine. Body thickest in the posterior quarter or third of its length; also slightly thickened near the anterior end; the middle region slender. Head up to about 0.45 mm. in diameter. Lips (Fig. 3) large, rather broader than long. Dorsal lip with two simple papillae, placed near the anterior margin. Ventro-lateral lips each with a lozenge-shaped papilla, with double termination, towards the ventral side, and a small, simple papilla towards the dorsal side, the latter nearly marginal. Interlabia massive, about half the length of the lips. Grooves at bases of lips well-developed. Denti-gerous ridges marginal, with large teeth, and with an indentation in the middle anteriorly. Oesophagus about 3 mm. long. Tail of male 0.25 mm. long, with a very short terminal spike. Spicules blunt at the tip, measuring 3.7 mm. in length, and, with the alae, 0.09 mm. in width. Postanal caudal papillae five

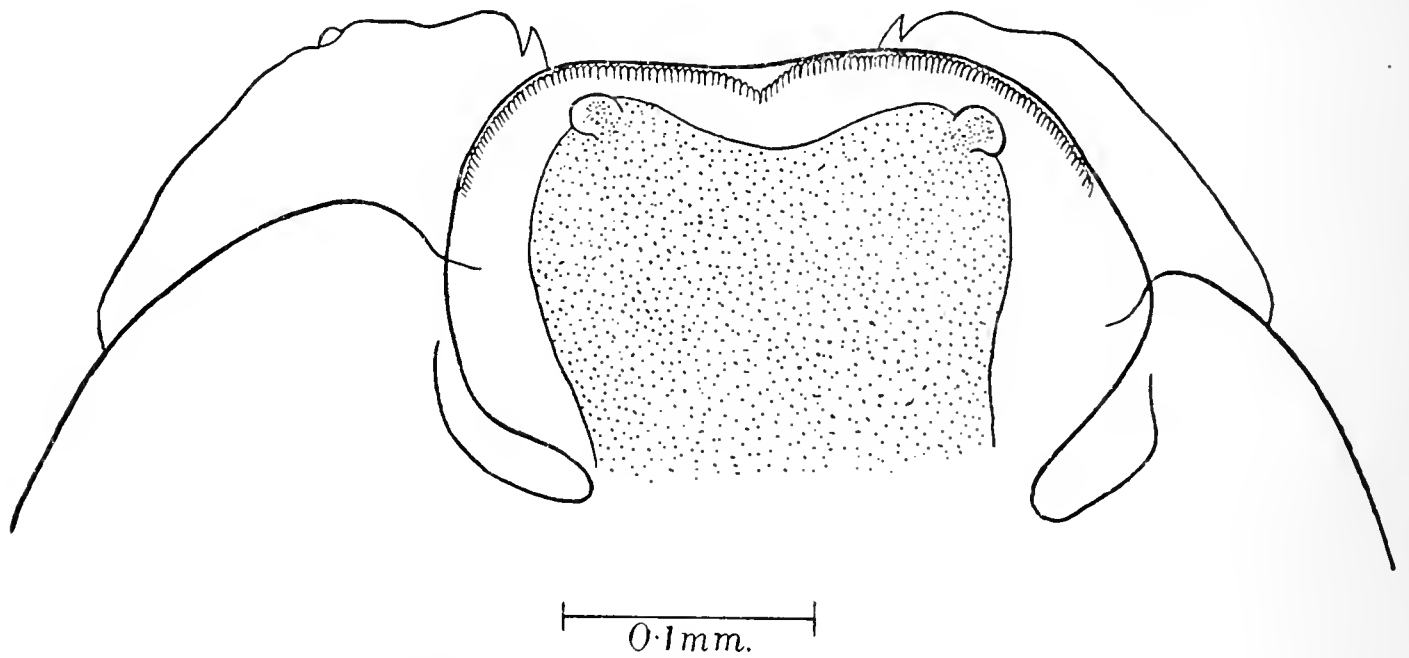


Fig. 3. *Ophidascaris mombasica*. The head; dorsal view.

pairs, of which four pairs form a group near the tip of the tail, two being large and lateral, two smaller and ventral. The papillae of the posterior ventral pair have double terminations. The fifth pair, which are widely separated from the rest, and lie near the corners of the cloacal aperture, are also double papillae, but not of very large size. Preanal papillae about 35 pairs, the intervals between them gradually becoming longer anteriorly. Tail of female very short (0.2 mm.) and blunt. Vulva situated at about the beginning of the posterior quarter of the body. Vagina runs forward a little, then doubles back, widening gradually into the uterus, which has two very wide branches running back almost to the tail. The coils of the ovarian tubes extend forward to a point a short distance in front of the vulva, and backward nearly to the posterior end of the body. Ova roundish-oval, measuring 0.0875–0.1 mm. \times 0.07–0.0825 mm.

HOST: *Psammophis subtaeniatus*.

LOCALITY: Mombasa.

The following forms probably also belong to this genus:

5. *Ophidascaris gestri* (Parona, 1890).*Ascaris gestri* Parona (1890), p. 768; Pl. III, figs. 7-8.

,, ,, Stossich (1896), p. 53.

Length, male, 55-67 mm.; female, 55-78 mm. Maximum thickness, 1 mm. Cuticular striations fine. Body elongate and slender, not tapering much at the extremities. Dorsal lip with two small rounded marginal papillae. Ventrolateral lips with two [? two each] small papillae on their margins anteriorly, and with an indentation on each side at their bases. Tail of female very short, with a terminal spike. Vulva in anterior third of body. [Uterus?] Tail of male with a terminal spike. Papillae small and sessile, two pairs postanal and ten pairs preanal. Spicules long, equal, with rounded tips.

HOST: *Tropidonotus piscator* [*T. quincunciatus*].

LOCALITY: Northern Tenasserim.

6. *Ophidascaris papillifera* (v. Linst., 1897).*Ascaris papillifera* v. Linstow (1897), p. 281; Pl. XXI, figs. 1-3.

Length, male, 38.2 mm.; female, 55 mm. Thickness, male, 0.75 mm.; female, 1.1 mm. Cuticular striations 0.039 [? 0.0039] mm. apart. Body much attenuated in front. Tail in both sexes very short and rounded. Lips almost circular, with marginal dentigerous ridges. Dorsal lip somewhat longer than broad, with narrow base, and bearing in front two large, oval, diagonally-placed papillae. Pulp with two lobules projecting forwards and inwards. Interlabia short, conical and rounded. Tail of male $\frac{1}{323}$ of total length. Six pairs of postanal papillae, of which one small pair is close to the cloaca and near the mid-ventral line, and one very large pair just behind these and more laterally placed. Preanal papillae 32-34 pairs, extending forward to 1.78 mm. from the caudal end, larger and closer together behind, becoming smaller and more widely spaced in front. Spicules 4.74 mm. long and 0.062 mm. wide. Tail of female $\frac{1}{324}$ of total length. [Vulva? Uterus?] Ova with very thick, closely stippled shell, measuring 0.073×0.065 mm.

HOSTS: Snakes (*indet.*).

LOCALITY: Bismarck Archipelago.

7. *Ophidascaris solitaria* (v. Linst., 1903).*Ascaris solitaria* v. Linstow (1903), p. 109; Pl. V, fig. 3.

Length (immature female), 44 mm. Thickness, 0.81 mm. Dorsal lip oval, broader than long (0.14 mm. \times 0.078 mm.). Dentigerous ridges nearly marginal. Interlabia low, pyramid-shaped. Oesophagus $\frac{1}{15}$ and conical tail $\frac{1}{259}$ of total length. [Vulva? Uterus?] Male unknown.

HOST: *Dipsadomorphus dendrophilus*.

LOCALITY: Siam.

8. *Ophidascaris naiae* (Gedoelst, 1916).*Ascaris naiae* Gedoelst (1916), p. 3.

Length, male, 62 mm.; female, 56.7 mm. Thickness, 1–1.07 mm. Cuticular striations $2.5\text{--}3\ \mu$ apart. Body cylindrical, more tapering in front than behind. Lips square, with rounded angles. Dorsal lip with two papillae, ventro-lateral lips each with one papilla. Dentigerous ridges present. Interlabia small, narrow, with rounded extremity, $\frac{1}{3}$ of length of lips. Oesophagus 2.9 mm. long, surrounded by the nerve-ring within its anterior fifth. Tail of male 0.2 mm. long, with six postanal papillae (four ventral, two sub-dorsal); preanal papillae 35 pairs. Spicules slightly unequal in length (5.04 mm. and 4.64 mm.), with rounded tips. Tail of female 0.24 mm. long, with conical tip. Vulva in middle of body. [Uterus?] Ova elliptical, with thick, finely punctate shell, measuring 0.08×0.072 mm.

HOST: *Naja nigricollis*.

LOCALITY: Belgian Congo.

9. *Ophidascaris intorta* (Gedoelst, 1916).*Ascaris intorta* Gedoelst (1916), p. 4.

Length (immature females) 100–110 mm. Thickness 0.736 mm. Cuticular striation not visible. Lips large, quadrangular, with minute, marginal dentigerous ridges. Interlabia small, triangular, with rounded extremity. Dorsal lip with two papillae, ventro-lateral lips with one each. Pulp with a flabelliform lobule directed forwards and outwards on either side, the free edge of which bears very numerous sharp digitations. Of these the outermost is the largest, and is directed backwards. Oesophagus $\frac{1}{27.6}$ of total length, surrounded by the nerve-ring within its anterior fifth. Excretory pore at 1.07 mm. from anterior end. Tail blunt, rounded, 0.18 mm. long, with terminal spike 0.03 mm. long. Vulva in anterior third of body. [Uterus?] Genital organs lie behind the level of the vulva.

HOST: *Bitis* sp.

LOCALITY: Belgian Congo.

(2) **Polydelphis** Duj., 1845.*Polydelphis* Dujardin (1845), p. 221 (as sub-genus of *Ascaris*).

GENERIC DIAGNOSIS. *Ascarinae*: Lips oblong, or more or less hexagonal, in outline, frequently longer than broad; usually broader at the base than at the free edge. Dorsal lip usually smaller than ventro-lateral lips. Two simple or double papillae on dorsal lip; one large simple or double papilla towards ventral side of each ventro-lateral lip. Pulp of ventro-lateral lips sometimes asymmetrical. Interlabia absent. No grooves at bases of lips. Oesophagus usually short, without bulb or ventriculus. A rudimentary intestinal caecum

sometimes present. Vulva usually in anterior region of body, rarely behind the middle. Vagina and uterus run posteriorly, the latter giving off either four or six parallel branches.

GENOTYPE: *P. anoura* Dujardin, 1845. (= *Ascaris* (*P.*) *pythonis* Retzius, of authors.)

The species may be divided into two sections, according to the number of uterine branches, as follows:

SECTION I. Forms with four-branched uterus.

1. *Polydelphis anoura* Duj., 1845.

Ascaris (*Polydelphis*) *anoura* Dujardin (1845), p. 221.

Ascaris attenuata Molin, 1858, of Stossich (1896), p. 77 (in part).

„ „ „ (♂) of v. Linstow (1899), p. 6.

„ *pythonis* Retzius, 1830, of Railliet and Henry (1910) (in part).

„ (*Polydelphis*) *pythonis* (Retzius, 1830) of Gedoelst (1916), p. 7.

Length, male, up to 116 mm.; female, up to 144 mm. Thickness, male, 2.15 mm.; female, 2.5–2.8 mm. Cuticular striations distinct, 4–10 μ apart. Body tapering in front, thick posteriorly. Lateral fields wide, more transparent than the rest of the cuticle. Lips small, rather longer than broad, nearly straight in front. Dorsal lip with two large double papillae; ventro-lateral lips each with one large double papilla towards the ventral side. Dentigerous ridges marginal, with minute teeth. Oesophagus¹ relatively long (10 mm.). No intestinal caecum. Tail of male blunt, 0.35 mm. long, with a small terminal spike. Spicules equal, conically pointed, 10.5 mm. long. Caudal papillae, two pairs postanal and 25 pairs preanal. Vulva at a little less than one-third of the total length from anterior end. Vagina passes gradually into the undivided portion of the uterus, which has a fusiform swelling about its middle. Length of this part of the uterus, together with vagina, about 30 mm. The four uterine branches relatively very short, originating in an oval swelling or reservoir, and running straight backward to end suddenly in narrow muscular canals about 3 mm. long. The latter are separated from the oviducts by globular or fusiform swellings. The four genital tubes bend forward soon after the termination of the uterine branches, the ovaries forming many coils about the intestine, and occupying the greater part of the posterior region of the body between vulva and anus. Ova nearly spherical, measuring 0.066–0.072 mm. in diameter.

HOSTS: *Python molurus*, *P. sebae*. Said also to occur in *Bitis arietans*, *Drymobius bifossatus*, [= *Coluber lichtensteini*], *Coluber corais*, *Zamenis constrictor*, ? *Coronella* [*Ophibolus*] sp.

GEOGRAPHICAL RANGE: India, Africa, ? America.

¹ Dujardin's statement that the oesophagus is followed by a narrow ventriculus seems to be erroneous.

Note on the name *P. anoura*.

This form does not appear to have been named by Retzius (1830), although it was first described by him. He refers to it merely as an *Ascaris* from *Python bivittatus*. Dujardin (1845), who re-described it, makes no reference to Retzius' description, but names it, as a new species, *anoura*. Railliet and Henry (1910) make the somewhat misleading statement that Retzius, in 1848, recognised his species as identical with *A. anoura* Dujardin, 1845. The fact appears to be that Creplin (1848) published a German translation of Retzius' description, adding footnotes of his own (signed "Cr."), in one of which he observes that Retzius' and Dujardin's species are identical, and appears to accept Dujardin's name. It seems, therefore, that the correct specific name of the form just described is *anoura*, and that the name *pythonis* has no claim to priority. How the apparently fictitious name *A. pythonis* has crept into the literature the writer is unable to discover. Possibly there has been some confusion between the *Ascaris* from a python and the *Bothriocephalus pythonis* described by Retzius in the same year and journal.

2. *Polydelphis attenuata* (Molin, 1858).

Ascaris attenuata Molin (1858), p. 147.

„ *rubicunda* (♀) Schneider (1866), p. 42.

„ *attenuata* Molin, 1858, of Stossich (1896), p. 77 (in part).

„ „ (♀) Molin, of v. Linstow (1899), p. 6.

„ *pythonis* Retzius, 1830, of Railliet and Henry (1910) (in part).

„ (*Polydelphis*) *attenuata* (Molin, 1858) of Geddoelst (1916), p. 5.

Length, male, up to 190 mm.; female, up to 258 mm. Thickness, male, up to 2 mm.; female, up to 3 mm. Cuticular striations $3\ \mu$ apart. Body tapering anteriorly, stout posteriorly. Lips (Fig. 4) small, oblong, somewhat longer than broad, with anterior border slightly emarginate and free angles rounded. Dorsal lip bears two papillae with double terminations, ventro-lateral lips each one double papilla towards the ventral side. Dentigerous ridges marginal, with very minute teeth. Oesophagus relatively long, $\frac{1}{11}$ to $\frac{1}{9}$ of total body-length. No intestinal caecum. Nerve-ring at about the anterior $\frac{1}{11}$ of oesophagus. Excretory pore a little behind nerve-ring. Tail of male conical, $\frac{1}{229}$ of total length. Spicules equal, 7–9 mm. long, with blunt tips. Caudal papillae, five pairs postanal (two sub-ventral, three lateral), 36 pairs preanal. Genital tube of male extends as far forward as the anterior third of the body. Posterior end of female very blunt, with small terminal spike. A pair of caudal papillae at 0.175 mm. from the extremity. Vulva at about the anterior fifth of the body. Vagina sinuous, widening gradually into the undivided portion of the uterus, from which it is not clearly marked off externally. The anterior part of the uterus runs straight back to a point about 35 mm. from the anterior end of the body, where it gives off four wide branches. These run straight back and

narrow suddenly behind into short muscular canals, about 1.5 mm. long, followed by small swellings of fusiform, oval or globular shape, apparently functioning as receptacula seminis. These mark off the uterine tubes from the oviducts, which, with the ovaries, are thrown into many convolutions, extending backwards nearly to the posterior end of the body, and then running forwards to a point about 5 mm. behind the vulva. Ova of roundish-oval shape, measuring 0.075–0.090 mm. \times 0.065–0.070 mm.

HOSTS: *Python molurus*, *P. sebae*, ? *P. reticulatus*, *Bitis arietans*.

GEOGRAPHICAL RANGE: India, Africa, ? Malay region.

3. *Polydelphis oculata* (v. Linst., 1899).

Ascaris oculata v. Linstow (1899), p. 6; Pl. I, fig. 7.

„ (*Polydelphis*) *oculata* Linst., 1907, of Railliet and Henry (1910).

Length, male, 58 mm.; female, 63 mm. Thickness, male, 2 mm.; female, 2.41 mm. Body tapering in anterior third, stout and cylindrical posteriorly.

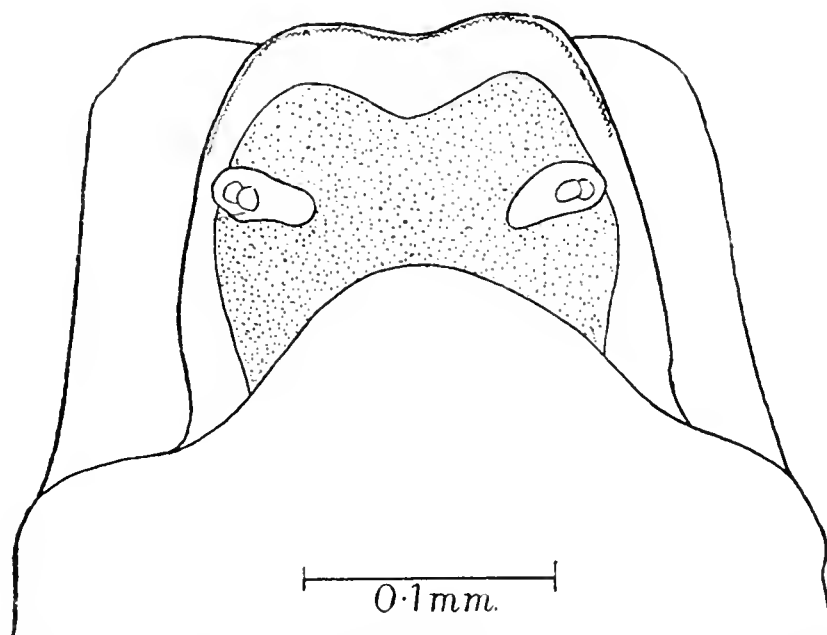


Fig. 4. *Polydelphis attenuata*. The head; dorsal view.

Lips nearly square, with free angles rounded. Dorsal lip slightly broader than long (0.18 \times 0.14 mm.). Cephalic papillae very large; two on dorsal lip. Each lobe of pulp of lip gives off an inward projection. Dentigerous ridges present. Oesophagus 6 mm. long. No intestinal caecum. Tail of male $\frac{1}{265}$ of total length, with a small terminal digitiform appendage. Spicules 5.4 mm. long. Caudal papillae, two pairs postanal, six pairs preanal. Tail of female $\frac{1}{53}$ of total length, blunt. Vulva at anterior third of body. Vagina at first simple, slender, sinuous, with a fusiform swelling near its origin, giving off, at 11 mm. from its origin, four wide branches 2 cm. long, lying in the same plane, and running backwards. Ova 0.060–0.067 mm. \times 0.055–0.060 mm. Embryos on hatching measure, according to Railliet and Henry, 0.4–0.425 mm. long.

HOSTS: *Python reticulatus*, *P. sebae*.

GEOGRAPHICAL RANGE: Malay region, Africa.

SECTION II. Forms with six-branched uterus.

1. *Polydelphis quadricornis* (Wedl, 1862).*Ascaris quadricornis* Wedl (1862), p. 469; Pl. II, figs. 17-19.

,, ,, Stossich (1896), p. 50.

? *Ascaris quadrangularis* Schneider (1866), p. 43; Text-fig.; Pl. I, fig. 10.

? ,, ,, Stossich (1896), p. 25.

? ,, *quadrilobata* v. Linstow (1908), p. 21; Pl. IV, fig. 1.

Although Wedl expressly states that this species has a four-branched uterus, the writer, after examining several sets of specimens which in other respects agree very closely with the characters given for *A. quadricornis*, is inclined to believe that the original description, in this particular, is faulty. Should this supposition ultimately prove to be incorrect, the form here described, which seems to be fairly common in the puff-adder, is probably a new species.

Size of mature specimens very variable. Length, male, up to 130 mm.; female, 70-155 mm. Thickness 2-3 mm. Body tapering in front, less so behind.

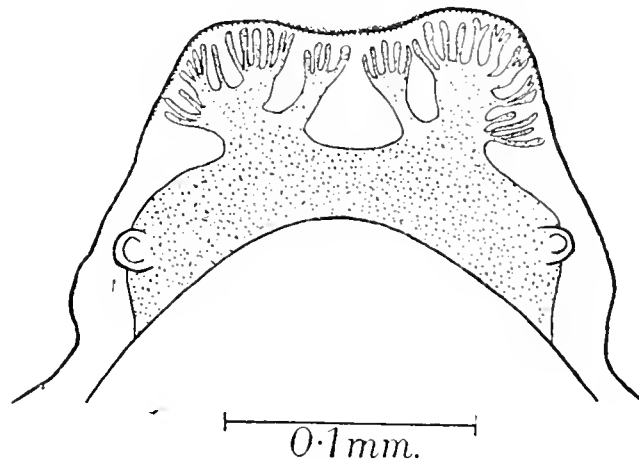


Fig 5. *Polydelphis quadricornis*. Dorsal lip, viewed from the exterior.

Lips somewhat hexagonal¹ in outline, broader at the base than at the free end. Dorsal lip (Fig. 5) with two small simple papillae near the lateral borders; ventro-lateral lips each with one large, lozenge-shaped papilla towards the ventral side. Pulp of dorsal lip sends out four lobes anteriorly, the outer lobe on each side large, the inner smaller. The outer lobes give off a large number of radiating processes, the inner lobes only four or five. Pulp of ventro-lateral lips asymmetrical, extending further forward laterally than ventrally; radiating processes present as in the dorsal lip. Dentigerous ridges marginal, with very small teeth. Oesophagus relatively short, about $\frac{1}{26}$ to $\frac{1}{18}$ of the total body-length. Many specimens have a short intestinal caecum running forward beside the base of the oesophagus, but this feature is apparently not constant. Tail in both sexes very short and rounded, with a small terminal spike. Tail of male with slight cuticular alae. Spicules short (about 1.75 mm.). Postanal papillae, five pairs—a group of four pairs near the tip of the tail (two lateral

¹ Wedl's figure of the lip (1862, Pl. II, fig. 19) indicates rather a square shape, but this may perhaps be due to the fact that, as stated, the lip is drawn from the inner aspect.

and two ventral) and a pair of large papillae, probably double, close behind and at the sides of the cloacal aperture. Preanal papillae at least 50 pairs. Vulva slightly in front of the middle of the body, in an annular constriction which is usually plainly visible to the naked eye. Vagina simple, muscular, about 4 mm. long in a 150 mm. specimen, passing into an undivided portion of the uterus, which gradually widens and, after a course of about another 4 mm., gives off six wide, thin-walled branches running back nearly straight for a distance of about 24 mm. These are followed by short, narrow, muscular canals, describing an S-shaped curve, and separated from the ovarian tubes by small oval swellings. The coils of the ovarian tubes extend backward nearly as far as the anus, and forward to the level of the vulva. In smaller specimens the measurements given for the female organs are, of course, proportionately smaller. Ova roundish-oval in shape, measuring 0.095–0.1 mm. \times 0.0875–0.0925 mm.¹, with shell 0.0075 mm. thick.

HOSTS: *Naja haje*, *N. nigricollis*, *Bitis arietans*, *Pseudaspis cana*, *Crotalus* sp.?

GEOGRAPHICAL RANGE: Africa, ? Brazil.

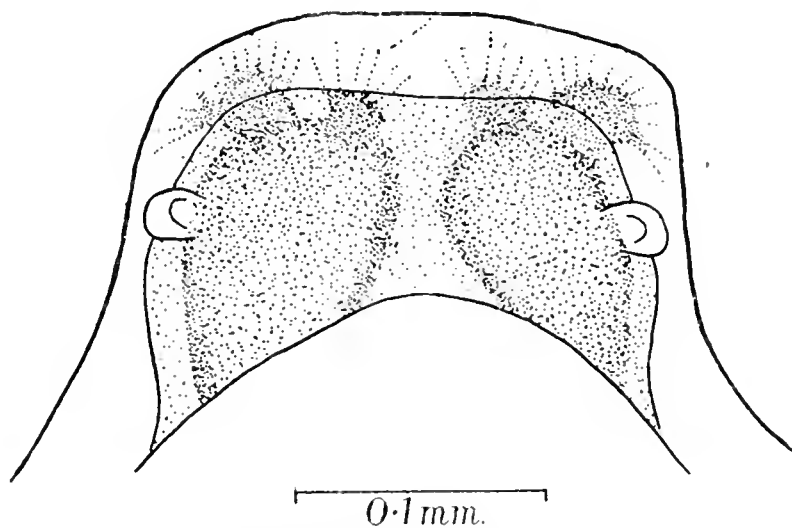


Fig. 6. *Polydelphis boddaertii*. Dorsal lip, viewed from the exterior.

2. *Polydelphis boddaertii* (Baird, 1860).

Ascaris boddaertii Baird (1860), p. 447.

„ „ Baird (1861), p. 229.

„ „ Örley (1882), p. 310.

„ „ Stossich (1896), p. 78.

Polydelphis boddaertii Baylis (1916), p. 416.

Length (female) 90 mm. Lips quadrangular, broader than long. Dorsal lip (Fig. 6) with two simple papillae; ventro-lateral lips each with one papilla. Pulp sends out radiating processes in front (vaguely-defined in the type-specimen), apparently from two main lobes on each side of the lip, the outer lobes being larger than the inner, as in *P. quadricornis*. Dentigerous ridges not seen. Oesophagus 4 mm. long. No intestinal caecum. Tail 0.4 mm. long, blunt, apparently without terminal spike. Vulva behind middle of body, dividing it in the proportion of 5 : 4. Vagina, together with undivided portion of uterus,

¹ 0.064 mm. \times 0.054 mm., according to Wedl.

about 7 mm. long, running posteriorly, and ending in an oval uterine chamber which gives off, laterally and posteriorly, six wide branches. These follow a sinuous course, parallel to each other, to within about 13 mm. from the posterior end of the body. The ovarian tubes turn forward shortly after their origin, and their coils extend anteriorly for a short distance in front of the vulva. Male unknown. Ova roundish-oval, measuring 0.088–0.095 mm. \times 0.075–0.080 mm., with shell about 0.007 mm. thick.

HOST: *Drymobius boddaertii*.

LOCALITY: West Indies.

3. *Polydelphis hexametra* (Gedoelst, 1916).

Ascaris hexametra Gedoelst (1916), p. 9.

Length, male, 51–55 mm.; female, 68.5–85 mm. Thickness, male, 1.3 mm.; female, 1.45 mm. Cuticular striation very fine, visible only under high powers. Body cylindrical, equally tapering at both ends. Lips sub-equal. Dorsal lip trapezoidal, with anterior angles rounded, bearing two lateral papillae. Ventro-lateral lips each with one papilla. Pulp undivided. Dentigerous ridges minute. Oesophagus $\frac{1}{22}$ to $\frac{1}{20}$ of total body-length. No intestinal caecum. Nerve-ring in anterior fifth or quarter of oesophagus. Excretory pore at 0.83–0.85 mm., and a pair of sessile cervical papillae at 0.975 mm., from the anterior extremity. Tail short, blunt, with small terminal spike. Tail of male with six pairs of postanal papillae—two pairs at about the anterior third of the tail (the more anterior of these sub-median); two large pairs at about $\frac{2}{3}$ of the length of the tail; one very small, sub-median pair and one larger, lateral pair a little in front of the tail-spike. Spicules unequal, measuring 0.91 mm. and 0.83 mm. in length, with rounded tips. Coils of male genital tube do not extend anteriorly beyond the middle of the body. Tail of female straight, $\frac{1}{152}$ of the body-length. Vulva in front of the middle of the body. Vagina, together with undivided portion of uterus, about 2.76 mm. long. Uterus gives off six branches 14 mm. long, continued as narrow muscular tubes, 0.4–0.45 mm. long, which pass into the oviducts. Most of the coils of the ovarian tubes extend backward to the posterior $\frac{1}{10}$ of the body, a few folds reaching forward to 1.9 mm. in front of the vulva. Ova nearly spherical, mean measurements 0.08 mm. \times 0.072 mm. Shell thick, with smooth [?] surface.

HOST: *Chamaeleon dilepis*.

LOCALITY: Belgian Congo.

4. *Polydelphis waterstoni*, sp.n.

Length (female) up to 110 mm. Thickness, 1.45 mm. Cuticular striation not apparent. Body slender, more tapering in front than behind. Diameter of head 0.3 mm. Lips rather broader than long, widest at the base, somewhat

hexagonal in outline, with straight anterior border. Dorsal lip with two simple lateral papillae; ventro-lateral lips each with one papilla towards the ventral side. Dentigerous ridges nearly marginal, with very small teeth. Oesophagus about $\frac{1}{27}$ of total length, with a maximum thickness (near the posterior end) of 0.35 mm. A short intestinal caecum present, about 0.4 mm. long, springing forward from a little behind the junction of oesophagus and intestine. Excretory pore at 0.95 mm., and nerve-ring at 0.8 mm., from the anterior end. A pair of cervical papillae, not prominent, just behind the nerve-ring. Vulva behind the middle of the body, dividing the total length in the proportion of 6 : 5. Vagina runs back as a muscular tube for about 3 mm. before widening into the uterus, which gives off six branches, running parallel to each other posteriorly. The coils of the ovarian tubes extend back to 13 mm. from the caudal end, and forward to a little behind the vulva. Ova nearly spherical, measuring 0.08–0.1 mm. in diameter. Male unknown.

Host: *Zamenis gemonensis*, var. *caspius*.

LOCALITY: Macedonia.

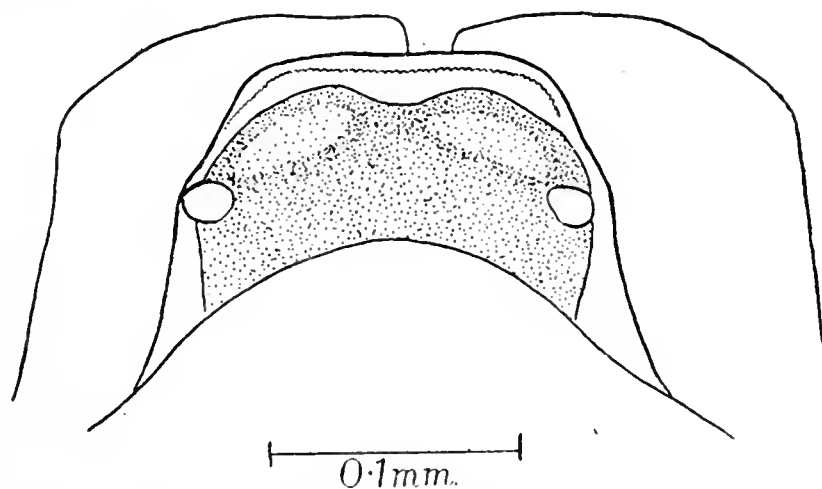


Fig. 7. *Polydelphis waterstoni*. The head; dorsal view.

NOTE. In the foregoing account no attempt has been made to give a complete list of the Ascarids of snakes. Some forms recorded under the name of *Ascaris* have been intentionally omitted, which cannot be assigned to either of the genera *Ophidascaris* and *Polydelphis*, or of which no description exists which is adequate for the determination of their position.

The nomenclature of the hosts has been, as far as possible, revised and corrected with the aid of Boulenger's Catalogue of the Snakes in the British Museum (1893–1896), and the writer's best thanks are also due to Dr Malcolm Smith for his help in this direction. Synonyms have been eliminated from among the names of hosts, except where it seemed an advantage to retain them, in which cases they have been enclosed in square brackets.

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ON THE CYSTS OF A HITHERTO UNDESCRIBED SPECIES OF *EIMERIA* IN HUMAN STOOLS.

By E. P. SNIJDERS.

(From the Pathological Laboratory, Medan, East Coast of Sumatra.)

(With 1 Text-figure.)

I.

MR C., who has lived for ten years in the tropics, was under the care of Dr de Jong and myself for chronic amoebic dysentery. Some five years ago he suffered from an abscess of the liver, readily cured after operation. Before this abscess he had never noticed any dysenteric symptoms, but afterwards he often had mild, or sometimes more severe, attacks of recurring dysentery, most times without fever. During the attacks, the bloody slime contained a great number of amoebae, principally of *Entamoeba histolytica* and containing erythrocytes. With an "emetine cure" the attacks regularly subsided; but between two attacks the patient produced constantly a great quantity of so-called "*minuta* forms" and 4-nucleate ("*tetragena*") cysts of this species in the faeculent parts of the stools. After an emetine treatment I often found a rather sudden increase in the number of cysts (always measuring from 11.5–13.5 μ), sometimes to a tremendous amount¹. Several times we tried to get rid of the amoebae by a cure with emetine bismuthous iodide, but without success, so the patient stayed under observation and regularly sent us his stools for control.

Now in the beginning of April, 1920, I received his stool once more, half-an-hour after he had passed it into a clean Petri dish. (This is our routine method to prevent contamination.) The stool was faeculent and pultaceous and contained no mucus or blood.

I found a few "*minuta* forms," but to my astonishment many round cysts, occurring uniformly in all parts of the faeces, but of larger dimensions than any that I had ever seen in human stools.

Without difficulty one could see that most of these cysts contained four whetstone-shaped structures strongly suggesting spores. These spores, in their turn, each enclosed two bodies—probably sporozoites; so on the whole I felt sure that I had the oöcysts of some species of *Eimeria* before me. Some of the oöcysts, however, were not completely differentiated; very few of them contained only one undifferentiated sphere showing a honeycomb structure, some others only two spores.

¹ Indeed for some time he formed my regular supply of cysts.

The oöcysts were colourless, transparent, and spherical. Their diameter was between 40μ and 48μ , most of them measuring 45μ . The capsule was, as far as could be seen, composed of two layers, quite transparent; the inner one being the proper, homogeneous, well-defined wall, the outer appearing as an ill-defined mucous layer which was often difficult to detect.

The whetstone-shaped spores had a length varying from $17-20\mu$ and a breadth of $7-8\mu$. The wall of the spore (sporocyst) was sharply defined except at certain spots on its outer surface where it appeared rough, possibly indicating the remains of a mucous outer layer. In general the ends of the spores were acute, but sometimes they were rather blunt.

It was rather difficult to see the form of the sporozoites, as will be clear from the drawings. They seemed to be rather slender, one end being more obtuse than the other, and to have these obtuse ends directed towards opposite poles. I could not detect nuclei with certainty and the "crystalline" small bodies observed by Dobell (1919) in *E. oxyspora* seemed to be absent.

Of an oöcystic residual body only vestiges seemed sometimes present. On the contrary, however, sporocystic residua were quite evident in the form of one or two highly refractile spherical bodies of varying, but rather small, size.

Sometimes the sporozoites had a "fixed," coagulated appearance, with some bursts, suggesting necrosis and disintegration of their coagulated bodies. Suspended in a 2 per cent. eosin solution (with 0.9 per cent. NaCl) these forms readily stained red, confirming the fact that these sporozoites were dead already.

Trying to obtain stained and durable preparations I met with difficulties. With the Heidenhain stain (after moist fixation in the warm fluid of Schaudinn) I did not succeed in obtaining any differentiation. One saw only the outline of the oöcysts and sometimes of the sporocysts, but nothing of their contents. In counterstaining with eosin, however, the bodies of the sporozoites stained red. But further details were not obtainable, at least not distinctly and constantly enough to rely on.

As of course these oöcysts puzzled me greatly, I afterwards made repeated examinations of this patient's stools, but I never succeeded in finding them again.

I may stipulate here that I have never found oöcysts of this kind in other patients, although I have made between 1000 and 2000 stool examinations a year for a period of six years, nor have I ever seen them in animal faeces. The only coccidial parasites I frequently meet with are those of *Eimeria stiedae* in rabbits and *Isospora bigemina* in cats¹.

Trying to identify this *Eimeria* I consulted Dobell's critical and exhaustive epitome on coccidia parasitic in man (1919), and for a moment thought it

¹ They are quite like the European forms; only it struck me that the dimensions of the cysts of *Eimeria stiedae* are smaller (length 30μ ($25-35\mu$), breadth $12-20\mu$) than is generally admitted in Europe.

was a specimen of his *Eimeria oxyspora*. A more thorough comparison, however, showed many differences:

1. The oöcysts are larger in my case (45μ as compared with 36μ).
2. The sporocysts are shorter ($17-20\mu$ as compared with $30-32\mu$).
3. The outer layer of the walls of oöcyst and sporocyst is rather indistinct.
4. The sporocystic residua are much more evident, and, on the contrary, an oöcystic residuum seems to be almost absent.
5. Dobell's "crystalline" bodies between the nucleus and the posterior end of the sporozoites are absent.

As for the cysts of *Eimeria wenyoni*, they also have a certain resemblance with the cysts here described; e.g. absence of oöcystic residuum, absence of the "crystalline" bodies, presence of highly refractile sporocystic residua, relative smoothness of the outer surface of the sporocystic wall. But undoubtedly the differences are more striking than the resemblances. Thus, in the first place, the cysts here described are more than twice as great in diameter (45μ as compared with 20μ); secondly, the spores are more slender, being of the same breadth but nearly twice as long as those of *Eimeria wenyoni*; thirdly, as a general rule, the ends of the sporocysts are acute and not obtuse; finally, the sporocystic residua are of a smaller size.

Moreover, it may be noted that I found in my case some undifferentiated and incompletely differentiated oöcysts in the stools, in contradistinction to the facts known of *Eimeria wenyoni* and *E. oxyspora*. But, with our present knowledge of this matter still being rather fragmentary, it is difficult to estimate the exact value of this difference.

Concerning the other coccidial parasites in man (hepatic *Eimeria* and *Isospora hominis*) I think confusion is impossible.

For all these reasons we are forced to accept the conclusion that the cysts in question belong to a species *hitherto undescribed in human stools*.

II.

Now if we find unexpected things in the stools we always have to reckon with three possibilities, and I think it a great mistake to omit consideration of any of them:

A. *The stool is contaminated after its deposition.* In our case I think this impossible, because the stool was passed into a dry, clean Petri dish; it was examined only half-an-hour later; and, above all, because we found the oöcysts thoroughly mixed up with the faecal mass and not only at its surface.

I have some reason for laying stress on this point, because I saw a case of a Javanese child wrongly diagnosed at the first examination as a case of Balantidiosis. This child was suffering from a severe attack of dysentery with bloody stools and high fever. In the stools the laboratory assistant found numerous protozoa, resembling *Balantidium*, many of which contained red blood-corpuscles, this being supposed an incontestable sign of pathogenicity. So it was proclaimed as a case of Balantidiosis.

When I saw the preparations it struck me that there were also a lot of small protozoa, probably of quite different species. Now it turned out that the pots used to be washed in a little river. Consequently, nearly each pot contained a small quantity of water abounding with protozoa, most of which belonged to the genus *Paramecium*. After adding to the preparation under the microscope a drop of a suspension of erythrocytes one saw that the peristomal cilia of the *Paramecia* caused a stream, by means of which the red corpuscles—amongst other small particles—were engulfed in the mouth.

A bacteriological examination of another motion of the child passed afterwards into a sterile Petri dish showed the bacilli of Shiga-Kruse.

B. *The cysts are ingested with food or water and pass unaltered (or only slightly altered) through the alimentary canal.* Thus they appear in the stools where eventually they may continue their development.

Such is, for example, the case with *Bodo* ("*Prowazekia*") cysts. On the East Coast I can get "*Prowazekia*" only out of the faeces of people who drink unfiltered water.

In the case here described I think this supposition rather speculative, though the fact that a certain number of cysts proved to be dead, might seem to support it. We would have then to accept, in the *first* place, a thorough pollution of water or certain food-stuffs with animal faeces (or possibly with other animal excretions), *e.g.* the faeces of aquatic animals, or of cockroaches, flies, ants, mice, or rats; in the *second* place, an abnormally good preservation of the great majority of the cysts on their passage through the alimentary canal of Mr C., *i.e.* a very remarkable resistance of the wall of the ripe oöcysts to the digestive action of the human gastric and pancreatic juices.

That these two conditions, however, should both be satisfied is rather improbable, especially as I have never met with similar cysts in the stools or intestines of the above-mentioned animals. Neither have I been able to find any reference, in the literature at my disposal¹, to an identical form in animals. The oöcysts of all other species are smaller, at least as far as the descriptions are clear enough to rely on and the dimensions given are exact.

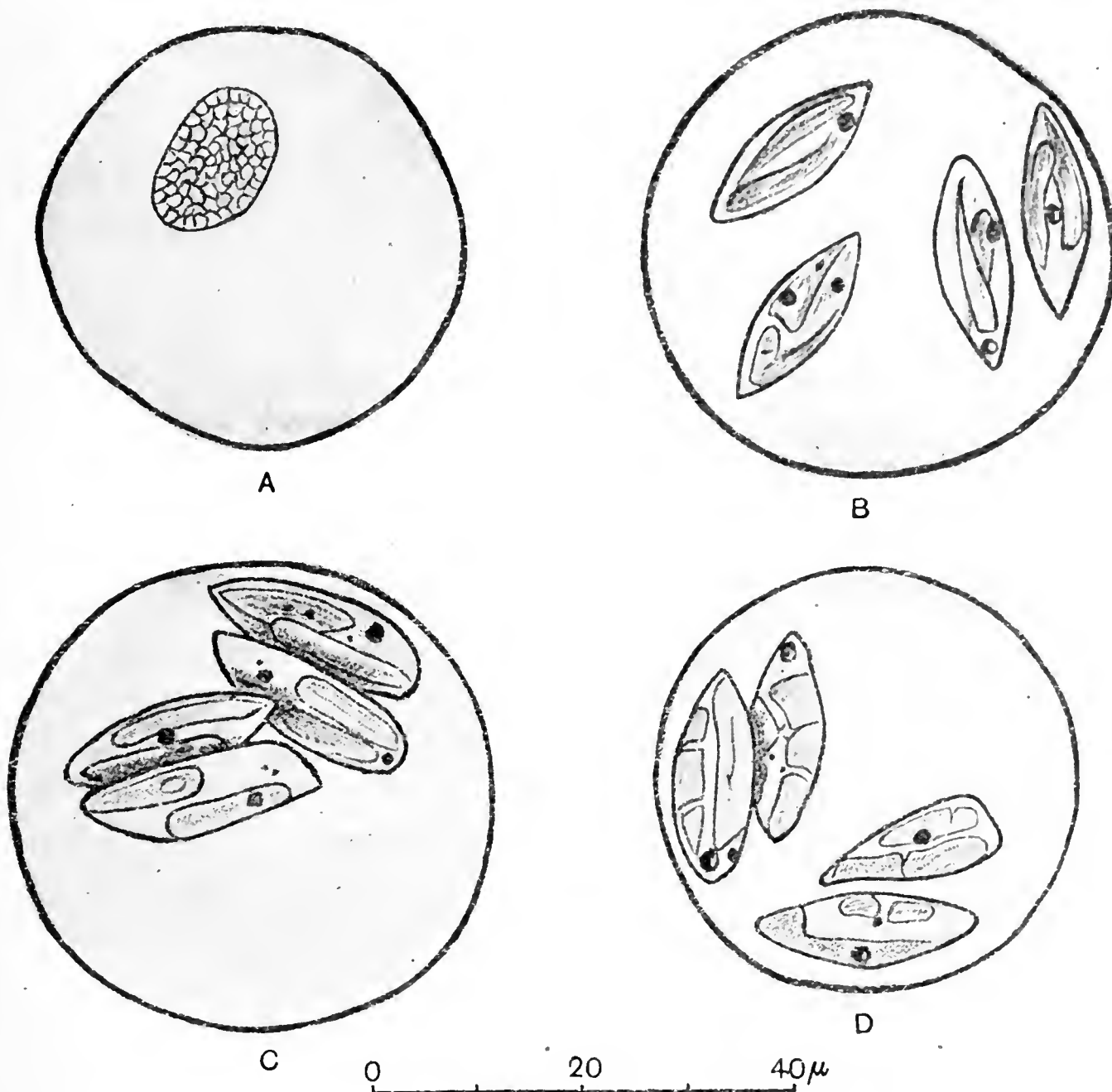
As to the second condition; of course it is not impossible that the wall of the oöcyst should be soluble in the digestive juices of the specific host only. This would represent then a high degree of physiological adaptation. But the facts known about the eggs of entozoa and the cysts of amoebae do not indicate the existence of such subtle differences in this respect.

But so far as I am aware we cannot deny this possibility (*water or food contamination*) altogether; in any case it is prudent to bear it in mind, and in certain cases it may prove to be worth a thorough investigation.

C. *The Eimeria is really parasitic in the described case, e.g. as a parasite of the epithelium of the small bowel.* (This last suggestion is probable because of the absence of liver-symptoms and because of the behaviour of *Eimeria* in

¹ The common Handbooks; *Arch. f. Protistenkunde*; *Centralbl. f. Bakt.*; *Parasitology*.

other animals.) If we accept this view, however, we must admit that its pathogenic power, if present, is of little importance, and either that the formation of an appreciable number of cysts very seldom occurs, or *that the infection is most transitory*.



The figures were drawn from the oöcysts as they appeared in a suspension of the fresh faeces in a 2 per cent. solution of cosin (with 0.9 per cent. NaCl). They are necessarily somewhat schematic. [The author's original figures (D excepted) show a faint line externally which delimits the cyst wall; this line unfortunately has been omitted from the figure as reproduced. The fine outer line should circumscribe the cyst at a distance equal to the thickness of the black contour.—Ed.] Magnification $\times 1000$.

- A. Oöcyst with undifferentiated sphere showing a honeycomb structure.
- B. Completely differentiated oöcyst, containing typical sporocysts and sporozoites.
- C. The same, but sporocysts and sporozoites somewhat irregular.
- D. Oöcyst with atypical sporozoites, looking "fixed," coagulated, and staining red with eosin.

If we now compare my case with those of Wenyon, Roche, and Dobell, we see that they are similar in the above-mentioned respects; and it should be noted that in most cases of *Isospora* infection also this absence of clinical symptoms and this short duration are circumstances almost constantly present.

So this sudden incidence and abrupt termination of the infection (or, at least, of cyst production), and the absence of clinical symptoms seem to be peculiar properties of the intestinal coccidial infections in man, and are certainly no obstacle to the acceptance of our third supposition.

Thus it may be concluded (though I must admit the impossibility of proving it to the hilt) *that the explanation which lies nearest to truth is that this case is one of parasitization of man by a hitherto undescribed Eimeria, which is probably peculiar to this host.*

The solution of the difficulties which we always meet with in rare infections, viz. the sources of infection, the modes of transmission, and the ways by which the infecting species preserves its continuity, must be reserved for further investigations.

MEDAN, *July* 1920.

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A NOTE ON THE NEW SPECIES OF *EIMERIA* FOUND IN MAN BY DR E. P. SNIJDERS.

BY CLIFFORD DOBELL, F.R.S.

IN the foregoing paper Dr E. P. Snijders has described the oöcysts of a species of *Eimeria* which he found in the stools of a patient under his care in Medan (Sumatra). The manuscript of this paper, together with a preparation containing the cysts in question, was kindly sent to me by the author¹ with the request that I would "be so kind as to add my opinion, and give to the species its right name." I shall do my best to comply with both these requests in the present note.

Dr Snijders has so fully described the cysts which he found, and has discussed their nature so ably, that there is little for me to add to his account. Careful examination of the specimen which he has sent me has suggested, however, a few points which appear worthy of note.

The specimen was a wet smear preparation of faeces, stained with iron haematoxylin and eosin and mounted in balsam. Dr Snijders describes it as "a bad one," but says he "could get no better." Nobody who has ever tried to make satisfactory permanent preparations of coccidial oöcysts and spores will be disposed to find fault with him on this score. It is frequently impossible to cause either fixatives or stains to penetrate these very resistant structures, and at present they can, in many cases, be studied properly in the fresh state only. I have never been able to obtain satisfactory stained preparations of any of the other coccidia of man.

On examining the preparation in question I was unable to recognize any oöcysts in it with certainty. I found, here and there, a few structures which appeared to be degenerate sporozoites, stained pink with eosin; but I was unable to make out the sporocysts or oöcysts enclosing them. I knew from experience, however, that the cysts of such organisms are often invisible when mounted in balsam—the cyst-walls and the balsam having approximately the same refractive index. I therefore removed the balsam with xylol, detached the cover-glass, and after passing the preparation through the various grades of alcohol, remounted the film in water. On re-examining it with a good lens, and suitably adjusted illumination, I was gratified to find a number of easily recognizable oöcysts—many of them, unfortunately, collapsed—containing

¹ I received the communication through the Editor of *Parasitology*: and I have therefore to thank Professor Nuttall not only for his good offices in this connexion but also for enabling me to publish this note simultaneously with Dr Snijders' paper.

spores whose walls (sporocysts) were quite intact. The pink-stained sporozoites within them appeared very degenerate, but were still recognizable. I made a number of careful measurements of the oöcysts and spores as they appeared in water. I then attempted to restain the preparation—with little success—and have remounted it permanently in euparal. In this medium, which has a lower refractive index than balsam, the cyst walls are barely visible.

I have had little difficulty in confirming Dr Snijders' statement that the cysts are those of a species of *Eimeria*. Every oöcyst contains the typical four spores, each of which—in all specimens sufficiently well preserved for study—encloses two sporozoites. Dr Snijders states that the diameter of the oöcysts is 40–48 μ . Most of those which I have seen are now too shrivelled for it to be possible to measure them accurately; but the few measurements which I have been able to make, and my estimates of the probable dimensions of the collapsed cysts, agree with his observations.

As regards the spores—which, as already noted, were still intact in many instances—I would note the following points. Dr Snijders states that they are whetstone-shaped, and measure 17–20 μ in length, with a breadth of 7–8 μ . They are shown best, I think, in his Fig. B: the more irregular outlines of Figs. C and D being due, apparently, to the fact that the spores in these were not all lying flat, but were seen more or less obliquely inclined to the axis of the microscope. It seems to me probable that Dr Snijders has not made sufficient allowance for this obliquity of many of the spores in making his measurements. At all events all my own measurements—made with great care from spores lying at right angles to the optical axis of the microscope, or estimated from those lying inclined to it at various angles—show that the length of the spores is slightly greater than he states. All my measurements lie between 20 μ and 25 μ , most of the spores measuring 22–24 μ . I find the width at the middle is—as stated by Dr Snijders—approximately 7–8 μ . (This dimension is not, of course, subject to the same error in its determination as the length.) All the spores appear to be fusiform, with their two ends equally pointed. Traces of an episporous ("mucous outer layer" of Dr Snijders) are visible on some; and the remains of sporocystic and oöcystic residual bodies can be made out in the majority of the cysts. It was impossible, however, to make out any details in the sporozoites.

It seems to me probable that the unsegmented oöcysts referred to by Dr Snijders, and shown in his Fig. A, are abnormal specimens. The protoplasmic inclusion appears far too small for a normal form in an early stage of development; but such abnormal stages are often seen in other species, and represent, I believe, dead oöcysts which have failed to get fertilized.

The oöcysts of this species are of unusually large size, and are certainly the largest described from man; but they are not the largest known in the Coccidia. Those of *Aggregata*, for example, attain much greater dimensions.

Although I was struck, at first, by the resemblance of these oöcysts to

those of *Eimeria oxyspora*, it seems clear, from their dimensions and those of their spores, that they belong to a distinct species. The dimensions of the spores in the Coccidia are very constant; and I do not know of any accurately described species which shows so great a range of variation in the form and size of its spores as that between *E. oxyspora* and the present organism. The relatively short spindle-shaped spores of the latter are quite distinct from the long whetstone-shaped spores of the former.

In my "Revision of the Coccidia parasitic in Man" (1919) I have discussed all the species of this group previously described: and Dr Snijders has pointed out that his *Eimeria* does not appear to belong to either of the intestinal species of this genus therein recognized—*E. wenyoni* and *E. oxyspora*. In this I fully agree; and I think Dr Snijders is to be congratulated, therefore, upon having demonstrated the existence of a hitherto undiscovered coccidium in man. There is a possibility, as he rightly points out, that the oöcysts may be those of an *Eimeria* belonging to some other host; but there is, I believe, no evidence at present in favour of such a supposition. It seems probable that the parasite is one which belongs to man himself. It is, however, somewhat remarkable that all the species of *Eimeria* hitherto found in human stools are not only very rare, but apparently cause infections which are peculiarly transitory. Their cysts have suddenly appeared in the stools and then promptly vanished—never to return.

Since I wrote my revision of the Coccidia of man, two papers purporting to describe new human cases of intestinal coccidiosis have appeared. The first, by Huetter (1919), records the finding of "coccidia" in sections of a rectal tumour from a woman: but even from the incomplete description of this case, it seems obvious that it was not really one of coccidiosis. Of the other case of "coccidiosis (?)" recorded by Lockhart-Mummery and Gabriel (1919) I can speak with more confidence. I have seen a preparation of the structures interpreted as coccidia, and have no hesitation in saying that they are either coccidia nor protozoa of any sort.

Drumpt (1918) has recently stated that the French Armies were infected to the extent of 0.2 to 0.33 per cent. with "*Eimeria (Coccidium)*": but—so far as I am aware—he has not described this organism. Chatton (1918, p. 218) states that he has found three cases of *Eimeria* infection in Southern Tunis, but does not name or describe the species. According to Mesnil (1919) it was probably *E. wenyoni*.

So far as I am aware, therefore, Dr Snijders' *Eimeria* belongs to a species which has not hitherto been described or named. I propose to call it *E. snijdersi*, in honour of its discoverer, and give the following diagnosis. For reasons already stated I have ventured to change the dimensions of the spores, as given by Dr Snijders, to those which I believe, from my own measurements of some of his specimens, to be correct.

Eimeria snijdersi nov. spec.

Oöcyst colourless, spherical, 40–48 μ in diameter. Spores fusiform, equally

pointed at both ends; length 20–25 μ , width in middle 7–8 μ . Oöcystic residue small, granular. Sporocystic residues in the form of one or two small refractile spheres. No “crystalline bodies”—like those of *E. oxyspora*—visible at the posterior ends of the sporozoites.

Habitat: intestine (?) of man.

As yet found in one case only, at Medan (Sumatra).

The structural characters given above serve to distinguish this species readily from the two other species of *Eimeria* previously described from human stools—*E. wenyoni* and *E. oxyspora*¹.

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October, 1920

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¹ I may note that M. Mesnil (1919) has, by an unfortunate misprint, inadvertently renamed this species “*E. oxyphila*.”

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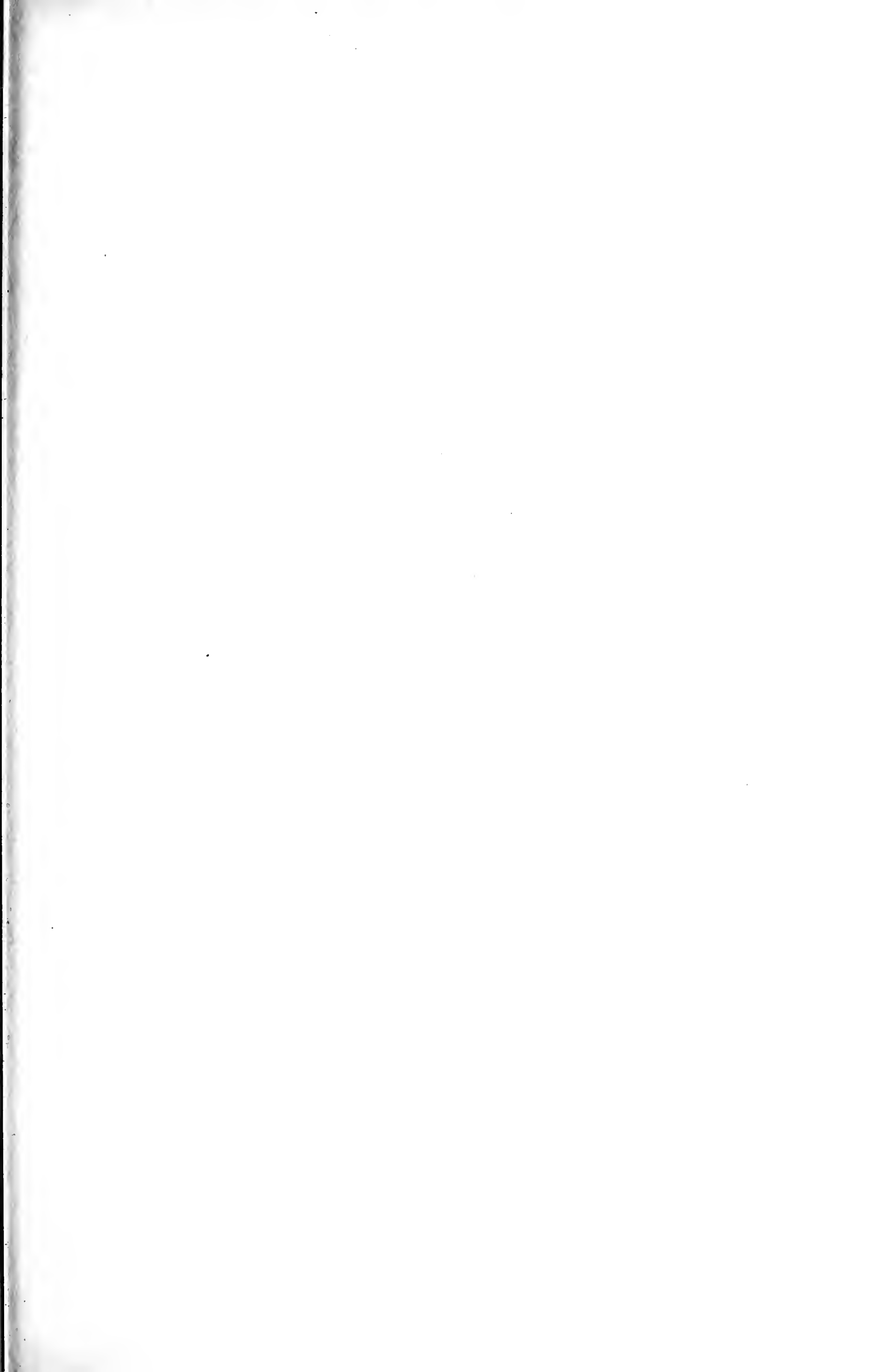
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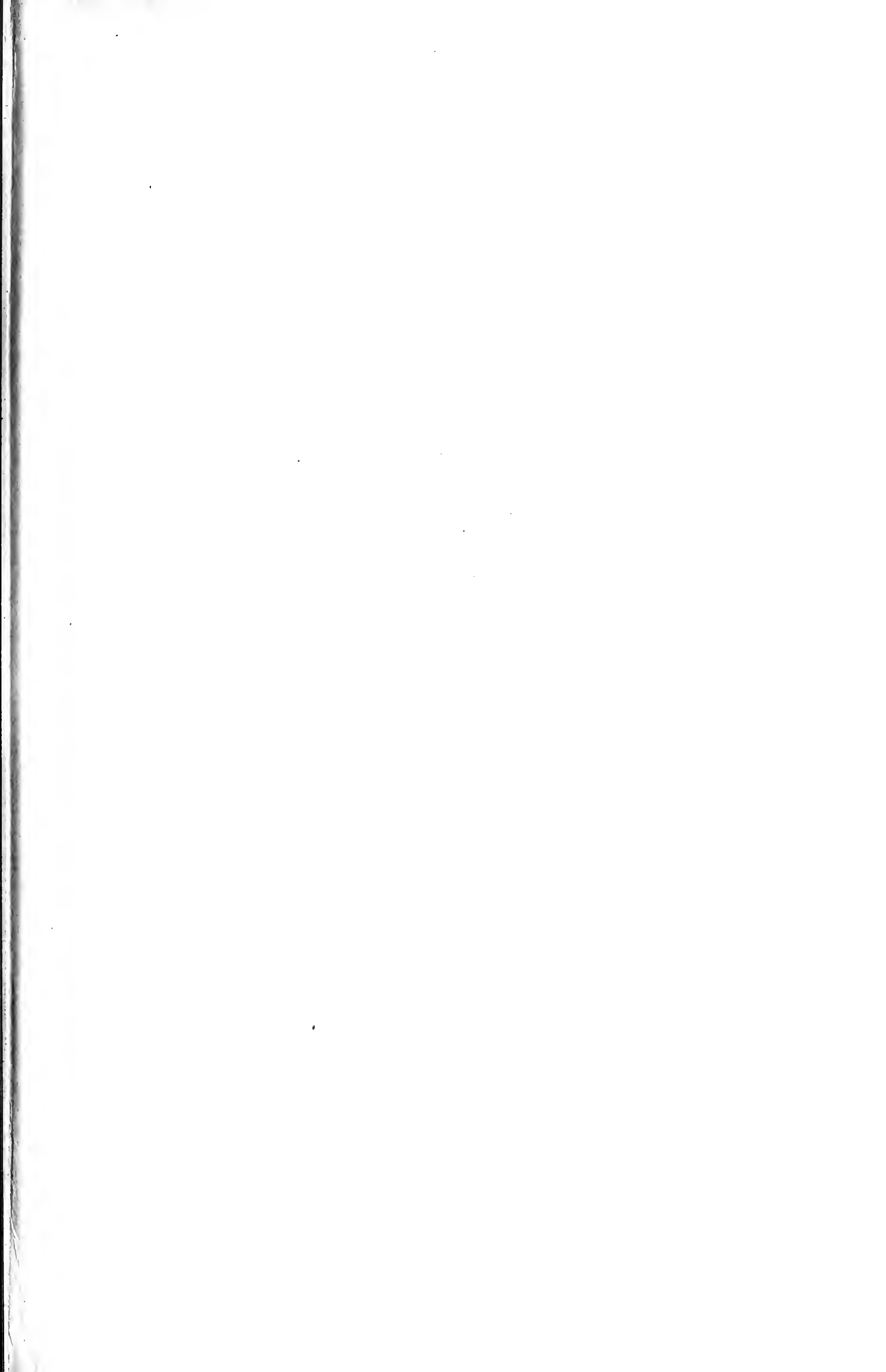
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